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Sexualhormone und Hemmstoffe
Sex Hormones and Inhibitors

Pharmacokinetics of Estradiol, Free and Total Estrone, in Young Women Following Single Intravenous and Oral Administration of 17β-Estradiol

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Summary

The pharmacokinetic parameters of estradiol (E_2, CAS 50-28-2), free and total estrone (E_1, CAS 53-16-7) were determined in 14 young women following a single oral administration of 2, 4 and 8 mg E_2 and a single intravenous administration of 0.3 mg E_2 in an open, intraindividual comparison with 4 treatments. The purpose of the study was to determine the absolute bioavailability of orally administered E_2 in a larger group of women and to assess the inter- and intraindividual variability of basic pharmacokinetic parameters of E_2 and metabolically derived E_1. In additon, the outcome of this study should provide a basis for the decision whether E_2 could potentially be used in a combination oral contraceptive.

There was a dose proportional increase in the AUC-values following the oral administration of 2 mg and 4 mg doses of E_2. At the high dose of 8 mg, however, only about 76 %, 78 % and 70 % of the expected values were found for E_2, free and total E_1, respectively. Especially the reduction in total E_1 concentrations points to an incomplete absorption of E_2 at the high dose level. The absolute bioavailability of orally administered E_2 was calculated based on the 4 mg dose and was found to be 4.9 ± 5.0 %. The mean ratio of free E_1 and E_2 concentrations in the serum, following parenteral and oral administration of E_2 was about 1.0 (i.v.) and between 8.8 to 19.8 (p.o.), respectively. Pharmacokinetic parameters, like AUC, derived from serum level-time curves of E_2, free and total E_1 showed a high intra- and interindividual variability. The AUC values calculated for total

E_1 were less variable than those calculated for E_2 and free E_1. Because of the very low bioavailability of oral E_2 on the one hand and the high inter- and intraindividual variability of estrogen levels in the serum on the other hand, E_2 seems not to be a likely alternative to ethinylestradiol as the estrogenic component in a combination oral contraceptive.

Zusammenfassung

Pharmakokinetik von Estradiol sowie freiem und gesamtem Estron nach einmaliger intravenöser und oraler Gabe von 17β-Estradiol an junge Frauen

Die pharmakokinetischen Parameter von Estradiol (E_2, CAS 50-28-2), freiem und gesamtem Estron (E_1, CAS 53-16-7) wurden in 14 jungen Frauen im Rahmen eines intraindividuellen Vergleichs mit 4 Behandlungen bestimmt. Estradiol wurde einmalig intravenös (0,3 mg) und oral in Dosen von 2, 4, und 8 mg verabreicht, wobei jeweils eine Auswaschphase von 3 Tagen zwischen zwei Behandlungen lag. Das Ziel der Studie war die Bestimmung der absoluten Bioverfügbarkeit von oral verabfolgtem E_2 und die Ermittlung der intra- und interindividuellen Varianz der pharmakokinetischen Parameter von E_2 und dessen Hauptmetaboliten E_1. Darüber hinaus sollten die Ergebnisse dieser Studie eine Grundlage für die Entscheidung liefern, ob E_2 möglicherweise als Bestandteil eines oralen Kontrazeptivums vom Kombinationstyp geeignet sein könnte.

Nach oraler Gabe von 2 und 4 mg E_2 wurde ein dosisproportionaler Anstieg der AUC Werte von E_2 und E_1 im Serum beobachtet. Bei der höchsten verabreichten Dosis von 8 mg wurden jedoch nur etwa 76 %, 78 % und 70 % der erwarteten Werte für E_2, freies und gesamtes E_1 gefunden. Insbesondere die Abnahme der Konzentration an gesamten E_1, deutet auf eine unvollständige Resorption von E_2 bei dieser Dosis hin. Die absolute Bioverfügbarkeit von E_2 nach oraler Gabe einer Dosis von 4 mg lag bei 4,9 ± 5,0 %. Das mittlere Konzentrationsverhältnis von freiem E_1 und E_2 im Serum nach parenteraler und oraler Applikation lag bei 1,0 (i.v.) bzw. im Bereich von 8,9 bis 19,8 (p.o.). Pharmakokinetische Parameter wie AUC, die aus den Konzentrations-Zeit Verläufen von E_2, freiem und gesamtem E_1 abgeleitet wurden, zeigten eine hohe intra- und interindividuelle Varianz. Die AUC Werte, die für gesamtes E_1 berechnet wurden erwiesen sich als weniger variabel als diejenigen für E_2 und freies E_1. Aufgrund der sehr niedrigen Bioverfügbarkeit von oral verabfolgtem E_2 einerseits und der hohen inter- und intraindividuellen Varianz der Estrogen-Spiegel andererseits, ist E_2 keine geeignete Alternative zu Ethinylestradiol als estrogener Komponente in oralen Kontrazeptiva.

Key words: CAS 50-28-2 · CAS 53-16-7 · Contraceptives, oral · Estradiol, bioavailability clinical pharmacokinetics · Estrone, bioavailability, clinical pharmacokinetics · Sex hormones

1. Introduction

Estradiol (E_2, CAS 50-28-2) ist the major estrogen in the endocrine regulation of humans and many other species. However, when administered orally, the steroid is subject to a substantial first pass effect, and the unchanged hormone becomes only to about 3 % bioavailable [1]. Although E_2 is completely absorbed over a wide dose range, there occurs a rapid and efficient biotransformation in the gut and the liver to a number of metabolites, mainly to estrone (E_1, CAS 53-16-7), which is subsequently conjugated to the corresponding sulfate and glucuronide, respectively [2, 3]. As a consequence of this high first pass effect, a previous study which was performed in only a few women, revealed a relatively large interindividual variance in the E_2 serum levels following oral administration [1]. the major source of information on the bioavailability and the metabolism of orally administered E_2 is provided by studies which have been performed in postmenopausal women. This is because E_2 and conjugated estrogens are widely used in hormone replacement therapy. Corresponding data from fertile, premenopausal women on the other hand, are scanty. However, for these women, the development of a combination oral contraceptive containing E_2 together with a progestogen could provide an attractive alternative to the conventional contraceptive preparations, which almost exclusively contain ethinylestradiol as the estrogenic component. A potential benefit of E_2 over ethinylestradiol could be the less pronounced effect of the former on hepatic parameters [4]. To guarantee contraceptive safety and reliable cycle control, however, sufficiently high, constant and reproducible E_2 concentrations have to be achieved after oral administration. Only if these minimum requirements are met, it would be worth considering the use of E_2 in a combination oral contraceptive. The limited information available so far on the pharmacokinetics of orally administered E_2 to young women, however, indicated that this might not represent a viable approach. For a proper judgement, however, more detailed and representative information on the bioavailability of E_2 as well as the inter- and intraindividual variability of E_2- and E_1-derived pharmacokinetic parameters is required with respect to the target population.

The aim of the present study was therefore to determine the absolute bioavailability of orally administered E_2 in a group of young women. Furthermore, by the administration of increasing oral doses of E_2, it should be investigated whether the serum levels of E_2 achieved in the women were linearly related to the dose. Finally, the inter- and intraindividual variability of several pharmacokinetic parameters of the administered E_2 and the metabolically derived E_1 was examined.

2. Material and methods

2.1. Study design

The study was designed as an open, intraindividual comparison with 4 treatments. The subjects were randomly allocated to one of the 3 treatment sequences (ABCD, ACDB, ADBC). The sequence of the 3 oral treatments was randomized according to a latin square. The first treatment was in all cases the intravenous administration.

Treatment A: intravenous administration of 0.3 mg E_2 dissolved in 1,2-propanediol/water (30/70);

Treatment B: oral administration of 2.0 mg micronized E_2 (with lactose in gelatine capsules);

Treatment C: oral administration of 4.0 mg micronized E_2 (with lactose in gelatine capsules);

Treatment D: oral administration of 8.0 mg micronized E_2 (with lactose in gelatine capsules).

The intravenously administered dose was given as a bolus (infusion time ca. 2 min), the oral doses were each administered together with 100 ml water. Between two treatments, there was a wash-out phase of 3 days.

Blood samples were taken prior to each drug administration and at the following time points thereafter:

Treatment A: 5, 15, 20, 30 and 45 min and 1, 1.5, 2, 3, 4, 6, 8,

Table 1: Demographic data of the participants.

Code no.	Initials	Age (years)	Weight (kg)	Height (cm)	Body surface (m^2)
1	S.M.	27	51.5	162	1.53
2	B.M.	32	58.7	161	1.61
3	G.K.	36	65.5	171	1.77
4	A.R.	21	70.6	176	1.86
5	R.S.	28	63.5	168	1.72
7	K.W.	23	60.5	168	1.69
8	A.W.	20	54.5	174	1.65
9	M.W.	34	64.0	174	1.77
10	M.A.	23	56.5	160	1.58
11	K.F.	22	66.0	187	1.89
12	A.G.	21	66.6	173	1.79
13	I.K.	35	69.0	171	1.81
14	G.M.	24	64.5	165	1.71
15	S.S.	26	57.0	163	1.61
Mean ± S.D.		27 6	62 6	170 7	1.71 0.11

12, 24 and 48 h;
Treatments B, C, D: 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 48 h.
All blood samples were kept at 4 °C until coagulation, the serum was separated and stored at -20 °C until analysis.

2.2. Study population

Fourteen healthy women whose demographic data are presented in Table 1, participated in the study. The subjects underwent a laboratory screening and a thorough medical and gynecological examination before entering the study. Excluded from participation were subjects who had any contraindication to the use of contraceptive steroids, or a concomitant medication which might interfere with the pharmacokinetics of E_2. Also excluded were women who smoked more than 20 cigarettes per day. One important inclusion criterion was the regular use of a combination oral contraceptive for at least one month, prior to as well as during this study. The intention of this criterion was to have as low as possible endogenous estrogen serum levels, which could interfere later with the analysis of the exogenously administered E_2. All participating women have been using different low-dose oral contraceptives prior to enrolment. One month prior to the administration of E_2, a preparation containing 0.15 mg levonorgestrel/0.03 mg ethinylestradiol (Microgynon®, Schering Aktiengesellschaft) was administered during a complete cycle. From day 5 of the subsequent treatment cycle onwards, single doses of E_2 were administered according to the treatment schedule.
The nature and purpose of the study were explained and written informed consent was given by each participant. The trial was approved by the local ethics committee.

2.3. Analytical methods

E_2 and E_1 concentrations in the serum were determined by specific radioimmunoassays. E_1 was analyzed with and without cleavage of conjugates.
E_2 concentrations in the serum were determined in duplicate following an extraction of 0.05 to 0.2 ml serum with 2.5 ml diethyl ether. A specific antiserum (immunogen: 17β-estradiol-6-CMO-BSA, Schering) was used at a dilution of 1 : 140 000 in the assay. The crossreaction with estrone and estriol was 7.4 and 0.3 %, respectively. Crossreactions against a large number of endogenous steroid hormones and contraceptive steroids were generally < 0.006 %. ^{3}H-estradiol (specific activity: 0.73 TBq/mmol; NEN Products, Boston, MA, USA) was used as tracer. Radiochemical purity was tested by HPLC and was > 98 %. Bound and free steroids were separated by charcoal treatment. The standards, which were also measured in duplicate, contained E_2 in buffer at a concentration range of 3.9–1000 pg/0.1 ml. The sensitivity of the standard curve was about 4 pg E_2 per tube, and the lower limit of quantification was 20 pg/ml. Inter-assay precision and accuracy were determined by the inclusion of five different control samples in each assay, containing a nominal concentration of 50, 200, 500, 1000 and 2000 pg E_2/ml serum, respectively. Experimentally measured concentration values were 45 ± 11 pg/ml, 231 ± 46 pg/ml, 695 ± 109 pg/ml, 994 ± 274 pg/ml and 2040 ± 344 pg/ml, respectively. The variation coefficient of inter-assay precision was between 16 and 28 %. Deviation of measured from nominal concentration values was between 2 and 39 %.
E_1 concentrations in the serum were determined in duplicate following an extraction of 0.1 to 0.2 ml serum with 2.5 ml diethyl ether. A specific antiserum (immunogen: estrone-6-CMO-BSA, Steranti Research Ltd, Hertfordshire, England) was used at a dilution of 1 : 10 000 in the assay. The cross-reaction with E_2 and estriol was 0.1 and 0.01 %, respectively. Cross-reactions against a number of endogenous steroid hormones were generally < 0.01 %. ^{3}H-[2.4.6.7]-estrone (specific activity: 4.0 TBq/mmol; NEN Products) was used as tracer. Radiochemical purity was tested by HPLC and was > 98 %. Bound and free steroids were separated by charcoal treatment. The standards, which were also measured in duplicate, contained E_1 in buffer at a concentration range of 3.9–1000 pg/0.1 ml. The sensitivity of the standard curve was about 4 pg E_1 per tube, and the lower limit of quantification was 20 pg/ml. Inter-assay precision and accuracy were determined by the inclusion of five different control samples in each assay, containing a nominal concentration of 50, 100, 200, 500 and 1000 pg E_1/ml serum, respectively. Experimentally measured concentration values were 45 ± 13 pg/ml, 129 ± 27 pg/ml, 169 ± 10 pg/ml, 473 ± 97 pg/ml and 923 ± 99 pg/ml, respectively. The variation coefficient of inter-assay precision was between 11 and 29 %. Deviation of measured from nominal concentration values was between 5 and 29 %.

2.4. Enzymatic cleavage of conjugates

0.4 ml serum were mixed with 0.1 ml sodium acetate buffer (pH 4.7, 0.1 mol, E. Merck, Darmstadt, FRG), additionally an enzyme mixture (glucuronidase 5 U/ml; sulfatase 14 U/ml; Boehringer Mannheim, Mannheim FRG) and a penicillin solution (10 IU) were added and the mixture was incubated for 12 to 18 h at 37 °C. At the end of the incubation period, the presence of active sulfatase was tested in representative samples by the addition of phenolphthaleinsulfate. The incubation mixture was extracted with 2.5 ml of diethyl ether, the residue was redissolved and used for the analysis of E_1, as described above.

2.5. Pharmacokinetic evaluation

The individual serum concentrations of E_2, free and total E_1 were used to derive the basic pharmacokinetic parameters following the parenteral and enteral administrations, respectively. Prior to data analysis, all concentration values were individually corrected for the endogenous levels of the respective hormone, by subtracting the corresponding pretreatment values from all subsequently measured values. The subtraction of an individually constant concentration value was deemed to be justified, since all individuals were using an oral contraceptive and should therefore have constant basal values of E_1 and E_2, respectively. This was supported by the fact that corresponding concentration values of E_1 and E_2, respectively, measured prior to each of the 4 administrations in each subject were comparable.
All calculations were performed model-free with the computer program TOPFIT (Goedecke, Schering AG, Thomae GmbH, FRG). The following parameters were calculated: Maximum drug concentrations in the serum (C_{max}) and the time when these were observed (t_{max}); area under the serum level-time curve (AUC), total clearance (CL) and volume of distribution (V); terminal half-life of disposition ($t_{1/2}$) and mean residence time (MRT).
In addition, following the parenteral administration of E_2, the concentration time curves of E_2 were evaluated by compartmental analysis (TOPFIT). For 4 out of 14 women, the data could be fitted by an open 3-compartment model, for another 5 women, an open two-compartment model was adequate.

3. Results

3.1. Intravenous administration of 0.3 mg E_2

The mean concentration values of E_2 measured in the serum of 14 women after intravenous administration of 0.3 mg E_2 are presented in Table 2 and Fig. 1. Maximum serum levels of 8321 ± 2434 pg/ml were observed already after 0.08 h. E_2 levels could be measured up to 48 h, typically up to 8 h, before the lower limit of quantitation was reached. The terminal half-life and the mean residence time were 1.7 ± 1.4 h and 0.7 ± 0.2 h, respectively.

Table 2: Concentrations (mean ± S.D.) of E_2, free and total E_1 in the serum of 14 women who received a single intravenous dose of 0.3 mg E_2. All concentration values were individually corrected for the pretreatment values.

Time of sampling (h)	E_2 (pg/ml)		E_1 free (pg/ml)		E_1 total (ng/ml)	
	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
0	0	0	0	0	0	0
0.08	8321	2434	960	339	1.6	0.7
0.25	3655	1509	796	435	2.3	1.4
0.33	2686	1075	638	318	2.6	1.5
0.5	1628	811	525	252	3.1	1.7
0.75	890	531	359	191	3.4	1.8
1	778	426	314	199	3.4	1.7
1.5	403	257	238	160	4.3	2.2
2	216	155	205	121	3.8	1.8
3	77	56	179	133	3.1	1.2
4	47	43	150	97	2.4	0.7
6	23	25	150	116	2.2	0.7
8	6	12	114	104	2.0	1.3
10	5	9	81	81	1.3	1.0
12	0	0	71	85	1.0	1.1
24	2	9	15	28	0.2	0.4
48	0	0	12	25	0.1	0.2

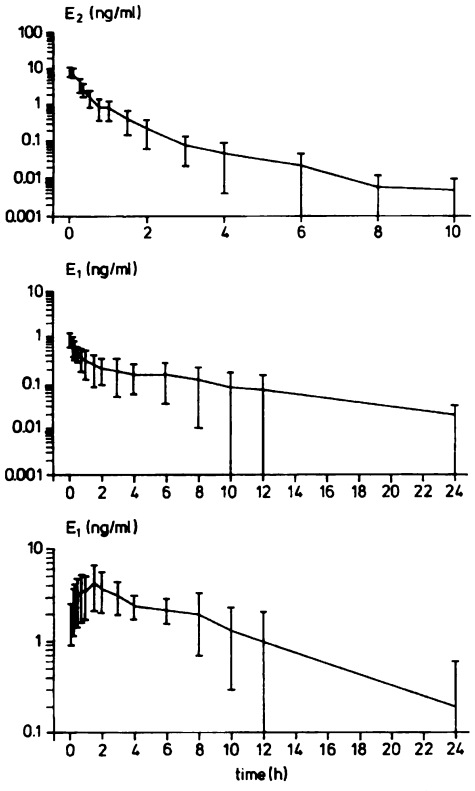

Fig. 1: Mean concentrations (± S.D.) of estradiol (*top*), free estrone (*middle*) and total estrone (*bottom*) in the serum of 14 women who received an intravenous administration of 0.3 mg estradiol. Both free and total estrone are major metabolites of estradiol.

Table 3: Pharmacokinetic parameters of E_2 (mean ± S.D.) following a single intravenous dose of 0.3 mg. The values were obtained by model-free calculation (n = 14), on the basis of an open 2-compartment (n = 5) or an open 3-compartment model (n = 4).

Parameter		Model-free	3-Compartment	2-Compartment
C_{max}	(pg/ml)	8321±2434	17250±3856	10718±2211
t_{max}	(h)	0.08±0.00	0.02±0.01	0.05±0.01
AUC (0–48 h)	(pg × ml^{-1} × h)	3265±1273	–	–
AUC	(pg × ml^{-1} × h)	–	3405±721	4010±1194
$t_{1/2}\,\lambda_1$	(h)	–	0.1±0.01	0.1±0.1
$t_{1/2}\,\lambda_2$	(h)	–	0.3±0.1	–
$t_{1/2}$	(h)	1.7±1.4	3.4±3.1	0.9±0.4
MRT	(h)	0.7±0.2	1.2±0.8	0.8±0.3
CL	(ml × min^{-1} × kg^{-1})	29.9±15.5	23.9±5.9	21.7±6.6
V_c	(l)	–	18.1±4.1	28.8±5.0
V_{ss}	(l)	72.8±23.7	100.3±52.5	63.7±19.7

Table 4: Pharmacokinetic parameters (mean ± S.D.) of free and total E_1, following a single intravenous administration of 0.3 mg E_2 to 14 women.

Parameter		E_1 free	E_1 total
C_{max}	(pg/ml)	1020±386	4700±2100
t_{max}	(h)	0.13±0.08	1.6±0.6
AUC (0–48 h)	(pg × ml^{-1} × h)	2899±2066	39500±27200
$t_{1/2}$	(h)	7.0±7.7	6.0±4.7
MRT	(h)	5.7±5.0	6.9±4.1

The total plasma clearance was calculated to be 29.9 ± 15.5 ml × min^{-1} × kg^{-1} and the volume of distribution (V_{ss}) was 72.8 ± 23.7 l. The pharmacokinetic parameters of E_2 which were calculated both model-free and based on compartmental analysis are presented in Table 3.

Mean concentrations of free and total E_1, measured following an i.v. dose of E_2, are presented in Table 2 and Fig. 1. Maximum concentrations of free E_1 of 1020 ± 386 pg/ml were observed already 0.13 h post administration and thus nearly coincided with the corresponding E_2 maximum. E_1 could be detected up to 48 h, typically up to 10 h post administration. The AUC(0–48 h) was 2899 ± 2066 pg × ml^{-1} × h and the terminal half-life and MRT were found to be 7.0 ± 7.7 h and 5.7 ± 5.0 h, respectively (Table 4). Maximum concentrations of total E_1 of 4.7 ± 2.1 ng/ml were observed 1.6 ± 0.6 h post administration. The AUC (0–48 h) was 39.5 ± 27.2 ng × ml^{-1} × h. A terminal half-life of 6.0 ± 4.7 h and a MRT of 6.9 ± 4.1 h were obtained (Table 4). Total E_1 could be measured up to 48 h, and in most cases up to 10 h, following the i.v. administration of E_2.

3.2. *Oral administration of 2 mg E_2*

The mean concentration values of E_2 measured in the serum of 14 women after single dose administration of 2.0 mg E_2 are presented in Table 5. E_2 profiles could be obtained only in 8 women, while no or only a few data

Table 5: Concentrations (mean ± S.D.) of E_2, free and total E_1 in the serum of 14 women who received a single oral dose of 2.0 mg E_2. All concentration values were individually corrected for the pretreatment values.

Time of sampling (h)	E_2 (pg/ml)		E_1 free (pg/ml)		E_1 total (ng/ml)	
	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
0	0	0	0	0	0	0
0.5	10	21	25	39	14.3	12.6
1	15	27	87	73	18.7	11.7
1.5	22	30	173	88	19.5	11.2
2	29	38	286	252	16.1	7.8
3	19	35	376	312	16.0	9.0
4	25	33	352	266	15.3	7.9
6	30	39	472	338	12.3	9.8
8	31	34	424	258	9.3	2.9
12	33	42	381	256	9.1	4.1
24	17	24	162	173	4.6	2.8
48	7	12	43	89	1.4	1.2

Table 6: Pharmacokinetic parameters (mean ± S.D.) of E_2, free and total E_1, following a single oral administration of 2.0 mg E_2 to 14 women.

Parameter		E_2	E_1 free	E_1 total
C_{max}	(pg/ml)	46±45	566±344	23400±9100
t_{max}	(h)	8.2±7.3	6.3±3.1	2.7±3.6
AUC (0–48 h)	(pg × ml^{-1} × h)	1043±1328	9960±7904	306100±152300
$t_{1/2}$	(h)	–	11.2±3.5	12.1±3.9
MRT	(h)	–	14.4±1.7	12.3±2.5

points were above the lower limit of detection in the remaining women. In the 14 women, maximum serum levels of 46 ± 45 pg/ml were observed 8.2 ± 7.3 h post administration and the AUC(0–48 h) was 1043 ± 1328 pg × ml^{-1} × h (Table 6).

The mean concentrations of free and total E_1 in the serum are presented in Table 5. Maximum concentrations of free E_1 of 566 ± 344 pg/ml were observed 6.3 ± 3.1 h after the oral administration of 2.0 mg E_2. The AUC(0–48 h) was 9960 ± 7904 pg × ml^{-1} × h. Terminal half-lives and mean residence times could be determined in 8 out of 14 women and were found to be 11.2 ± 3.5 h and 14.4 ± 1.7 h, respectively (Table 6).

The maximum concentrations of total E_1 were observed 2.7 ± 3.6 h after drug intake and amounted to 23.4 ± 9.1 ng/ml. The AUC (0–48 h) was 306.1 ± 152.3 ng × ml^{-1} × h. Mean terminal half-life and MRT were found to be 12.1 ± 3.9 h and 12.3 ± 2.5 h, respectively (Table 6).

3.3. Oral administration of 4 mg E_2

The mean concentration values of E_2, free and total E_1 measured in the serum of 14 women are presented in Table 7 and Fig. 2. Maximum concentrations of E_2 were

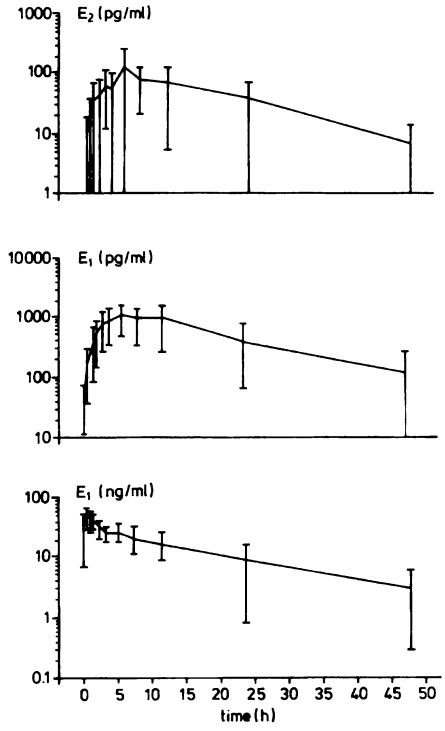

Fig. 2: Mean concentrations (± S.D.) of estradiol (*top*), free estrone (*middle*) and total estrone (*bottom*) in the serum of 14 women who received an oral administration of 4.0 mg estradiol. Both free and total estrone are major metabolites of estradiol.

Table 8: Pharmacokinetic parameters (mean ± S.D.) of E_2, free and total E_1, following a single oral administration of 4.0 mg E_2 to 14 women.

Parameter		E_2	E_1 free	E_1 total
C_{max}	(pg/ml)	163±216	1170±696	53000±13000
t_{max}	(h)	6.5±3.0	6.9±3.2	1.2±0.6
AUC (0–48 h)	(pg × ml^{-1} × h)	2290±2090	23191±16380	591000±307000
$t_{1/2}$	(h)	13.5±4.4	14.1±3.7	10.8±3.8
MRT	(h)	18.7±8.2	15.3±2.1	11.7±3.1

observed 6.5 ± 3.0 h following an oral administration of 4 mg E_2 and amounted to 163 ± 216 pg/ml. An AUC (0–48 h) value of 2290 ± 2090 pg × ml^{-1} × h was calculated and the mean terminal half-life and the MRT were 13.5 ± 4.4 h and 18.7 ± 8.2 h, respectively (Table 8).

Table 7: Concentrations (mean ± S.D.) of E_2, free and total E_1 in the serum of 14 women who received a single oral dose of 4.0 mg E_2. All concentration values were individually corrected for the pretreatment values.

Time of sampling (h)	E_2 (pg/ml)		E_1 free (pg/ml)		E_1 total (ng/ml)	
	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
0	0	0	0	0	0	0
0.5	9	14	35	38	29.0	22.2
1	19	23	169	135	48.1	17.2
1.5	37	39	365	289	44.5	15.6
2	41	48	477	335	40.6	9.6
3	66	53	780	494	30.7	10.5
4	57	57	839	515	25.3	7.1
6	147	220	1022	599	25.9	10.4
8	84	55	888	564	21.0	9.4
12	86	76	929	677	16.8	8.4
24	38	50	386	329	9.1	8.3
48	7	11	117	134	3.3	3.0

Table 9: Concentrations (mean ± S.D.) of E_2, free and total E_1 in the serum of 14 women who received a single oral dose of 8.0 mg E_2. All concentration values were individually corrected for the pretreatment values.

Time of sampling (h)	E_2 (pg/ml)		E_1 free (pg/ml)		E_1 total (ng/ml)	
	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
0	0	0	0	0	0	0
0.5	27	41	131	104	41.8	28.6
1	46	44	420	352	61.2	4.7
1.5	80	54	705	580	54.1	24.1
2	93	56	955	727	54.9	7.9
3	111	75	1294	1214	50.9	11.9
4	126	93	1216	1100	42.2	10.9
6	141	109	1349	1009	36.3	14.0
8	128	104	1284	1044	29.8	9.5
12	125	84	1211	1109	24.9	10.9
24	53	48	563	486	13.5	8.3
48	18	18	197	217	3.3	2.6

Table 10: Pharmacokinetic parameters (mean ± S.D.) of E_2, free and total E_1, following a single oral administration of 8.0 mg E_2 to 14 women.

Parameter		E_2	E_1 free	E_1 total
C_{max}	(pg/ml)	171±106	1641±1221	66500±11700
t_{max}	(h)	6.7±3.6	6.0±3.3	1.8±0.9
AUC (0–48 h)	(pg × ml^{-1} × h)	3278±2287	33181±27557	840000±305000
$t_{1/2}$	(h)	15.0±6.1	15.8±5.4	12.6±3.9
MRT	(h)	20.4±10.3	15.4±2.1	12.5±2.1

Maximum concentrations of free E_1 of 1170 ± 696 pg/ml were found at 6.9 ± 3.2 h post dose. The AUC(0–48 h) was 23 191 ± 16 380 pg × ml^{-1} × h, and mean terminal half-life and MRT values were 14.1 ± 3.7 h and 15.3 ± 2.1 h, respectively (Table 8).

For total E_1, maximum concentrations of 53.0 ± 13.0 ng/ml were observed 1.2 ± 0.6 h post administration. The AUC(0–48 h) was 591 ± 307 ng × ml^{-1} × h, and for the terminal half-life and the MRT, mean values of 10.8 ± 3.8 h and 11.7 ± 3.1 h were found, respectively (Table 8).

3.4. Oral administration of 8 mg E_2

The mean concentration values of E_2, free and total E_1 measured in the serum of 14 women are presented in Table 9. Maximum concentrations of E_2 were observed 6.7 ± 3.6 h following an oral administration of 8 mg E_2 and amounted to 171 ± 106 pg/ml. An AUC (0–48 h) value of 3278 ± 2287 pg × ml^{-1} × h was calculated and the mean terminal half-life and the MRT were 15.0 ± 6.1 h and 20.4 ± 10.3 h, respectively (Table 10). The maximum concentrations of free E_1 of 1641 ± 1221 pg/ml were measured at 6.0 ± 3.3 h post dose. The AUC (0–48 h) was 33 181 ± 27 557 pg × ml^{-1} × h, and the mean terminal half-life and MRT values were 15.8 ± 5.4 and 15.4 ± 2.1 h, respectively (Table 10).

For total E_1, maximum concentrations in the serum of 66.5 ± 11.7 ng/ml were observed 1.8 ± 0.9 h post administration. The AUC (0–48h) was 840 ± 305 ng × ml^{-1} × h, and for the terminal half-life and the MRT, mean values of 12.6 ± 3.9 h and 12.5 ± 2.1 h were found, respectively (Table 10).

3.5. Absolute bioavailability of E_2

For the calculation of the absolute bioavailability of orally administered E_2, principally all three oral doses can be considered and related to the intravenous administration. Thus, the absolute bioavailability of E_2 was found to be 5.5 ± 9.2 %, 4.9 ± 5.0 % and 3.3 ± 1.8 % based on orally administered doses of 2.0 mg, 4.0 mg and 8.0 mg, respectively.

3.6. Dose dependence of serum levels of E_2, free and total E_1, following the oral administration of 2, 4 and 8 mg E_2

When the AUC(0–48 h) values of E_2, free and total E_1 were examined under the aspect of dose dependence, all three parameters revealed the same trend. Although there was a dose proportional increase in the AUC values following the administration of the 2.0 mg and the 4.0 mg doses, at the high dose of 8.0 mg, only about 76 %, 78 % and 70 % of the expected values were found for E_2, free and total E_1, respectively (Fig. 3). Especially the reduction in total E_1 concentrations points to an incomplete absorption of E_2 at the high dose level, and accordingly, the serum levels of E_2 and free E_1 were less than anticipated. For that reason, the calculation of the absolute bioavailability of E_2 should be based on the 4.0 mg dose only, since follwing administration of the lower

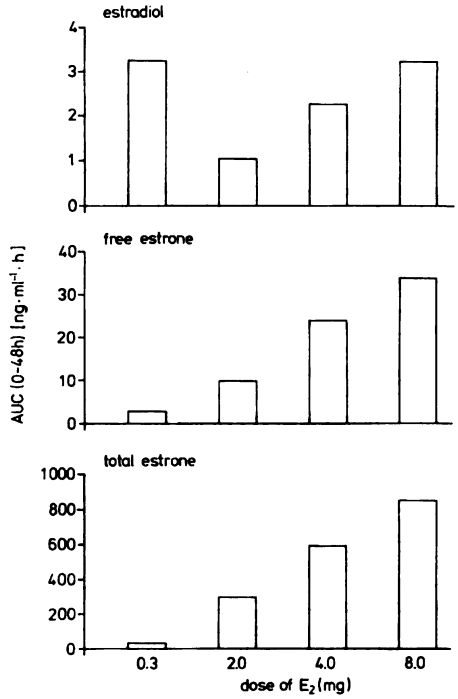

Fig. 3: Dose dependence of the AUC(0–48) values (mean) of estradiol (*top*), free estrone (*middle*) and total estrone (*bottom*) following single dose administration of 0.3 mg estradiol (i.v.), 2, 4 and 8 mg estradiol (p.o.), respectively.

dose, E_2 could only be measured in 8 out of 14 women, and after the high dose, absorption was obviously incomplete.

3.7. Ratio of free E_1 and E_2 concentrations in the serum, following parenteral and oral administration of E_2, respectively

The mean E_1/E_2-concentration ratio after the intravenous administration of E_2 observed in the serum was 1.0 ± 0.6. The ratio increased to values of 8.8 ± 8.5, 19.8 ± 18.3 and 12.9 ± 12.2 following the oral administration of E_2 in doses of 2, 4 and 8 mg, respectively.

4. Discussion

The purpose of the present study was to measure the serum levels of E_1 and E_2, and to assess the pharmacokinetic parameters of both steroids following parenteral and oral administration of E_2 to premenopausal women. In order to have only minimal interference from endogenous estrogens, the participants were under oral contraceptive therapy throughout the study. This should result in suppressed endogenous E_1 and E_2 levels, due to the central action of the contraceptive steroids on the hypothalamic-pituitary-ovarian axis. In fact, in the majority of women, pretreatment values of E_2 were below the lower limit of quantification, while in the remaining women concentrations in the range of ca. 25 to 60 pg/ml were measured in the serum. Similarly, pretreatment serum levels of E_1 were below the detection limit in most women, in others, however, concentrations well above this limit were measured. Following enzymatic cleavage of the conjugates, there were only a few women where E_1 could not be detected in the serum, most women had serum levels in the range of about 0.2 to 2 ng/ml prior to drug administration. Therefore, it was assumed that the individual pretreatment levels of both E_1 and E_2 remained constant throughout the observation period and were therefore subtracted from all corresponding concentration values measured, following the exogenous administration of E_2. This procedure seemed justified since all women were regularly taking their oral contraceptive preparation throughout the whole study and thus their individual estrogen levels should remain at a relatively constant value.

Following the intravenous administration of 0.3 mg E_2, either a biphasic or a triphasic disposition patern of E_2 was observed in the serum. The initial disposition phases (one in the 2-compartment and two in the 3-compartment model) were in the range of 0.1 to 0.3 h. The terminal phase was characterized by mean half-lives of 0.9 h and 3.4 h in those women whose serum level time curves could be fitted by either a 2- or a 3- compartment model and a mean value of 1.7 h was calculated for all women based on a non-compartment-dependent analysis. Thus, a rapid elimination of E_2 from the serum was observed, which was also evident by the high clearance rates of about 22–30 ml × min^{-1} × kg^{-1}. As compared to a hepatic serum flow of about 12 ml × min^{-1}, and an E_2 clearance of about 11 ml × min^{-1} × kg^{-1} determined in previously performed studies in postmenopausal women, clearance was about 2–3 times higher in the present study [1, 5]. This could suggest that in the present study, hepatic clearance of E_2 might have been increased due to the concomitant administration of an oral contraceptive. In fact, it has been reported that synthetic progestogens can induce the 17β-hydroxysteroid dehydrogenase (HSDH) and lead to an increase in the metabolic clearance rate of E_2 [6]. In a clinical study, however, where several estrogens were administered orally in the presence and in the absence of 0.25 mg levonorgestrel, such a clear-cut effect on the serum levels of free and conjugated E_1 and E_2 was not observed [7]. A total clearance value exceeding the hepatic serum flow could be an indication of a contribution of extrahepatic metabolism to the total clearance. On the other hand, there is a large interindividual variation in the pharmacokinetic parameters of E_2 and this might well account for the different results obtained in the present study as compared to the previous studies.

Following the intravenous administration of E_2, maximum concentrations of free E_1 and E_2 in the serum were reached at about the same time. Compartment-independent analysis in all 14 women, revealed a mean terminal half-life of 7 h and a corresponding MRT of 6 h. Thus the terminal disposition half-life of E_1 was about 4 times longer and the MRT about 9 times longer than the values found for E_2. The E_1/E_2-concentration ratio following i.v. administration of E_2 was around unity, which is equivalent to the reported ratio of the endogenous estrogens in fertile females [8] and to the ratio observed following transdermal administration of E_2 [9].

For total E_1 concentrations, maximum values were observed about 1.6 h post administration, and both terminal half-life (ca. 6 h) and MRT (ca. 7 h), were practically identical to those observed for free E_1. The mean E_1-total/E_1-free concentration ratio was about 19, underlining the importance of E_1-sulfate as the main pool of circulating E_1. Similar or somewhat less values have been reported earlier for women who had received a parenteral administration of E_2 and this seems also to represent the endogenous concentration ratio in women [1].

Following the oral administration of E_2 in doses of 2, 4 and 8 mg, a dose-dependent increase in E_2 serum levels was observed in most women. However, at the lowest dose of 2 mg, basal E_2 serum levels remained practically unchanged in 7 women, and only minor changes were seen in the other women. At higher doses of E_2, in particular with the 8 mg dose, marked increases in E_2 serum concentrations were seen after drug administration. These results are in good agreement with those reported by others [10]. A linear and dose proportional relation of dose of E_2 administered and AUC(0–48 h) calculated was observed between the 2 mg and the 4 mg dose. At the high dose of 8 mg, however, a value was obtained which, under the assumption of dose proportionality, was only about 77 % of the expected AUC-value. Similar dose-AUC-relations were found for free and total E_1, which indicates an incomplete absorption of E_2 at the highest dose. It is well known that unconjugated E_1 and E_2 are only poorly absorbed from the gastrointestinal tract. Some improvement in the extent of absorption has been achieved by the use of micronized E_2 or by the administration of conjugated estrogens [3, 10]. In the present study, micronized E_2 was administerd in gelatine capsules which allowed for a rapid release of the drug in the gastrointestinal tract. The observed impaired absorption at a dose of 8 mg E_2 is therefore not due to the formulation administered.

The absolute bioavailability of E_2 was calculated to be about 5 %, which is in good agreement with the results of previous studies [1]. It has been shown by others that a considerable part of the dose administered is already metabolized in the gastrointestinal mucosa and that E_1 is the major metabolite formed [2, 3]. Following an oral dose of E_2, a much higher ratio of free E_1/E_2 (8 to 20) was observed as compared to the intravenous dose, where this ratio was close to unity. The high E_1/E_2-ratios are probably mainly a result of the high concentration of E_2, reaching the liver via the portal vein and the high metabolic capacity of the liver. This preponderance of E_1 has been confirmed meanwhile in a number of studies, and a E_1/E_2 ratio of about 5 has been reported after the oral administration of 2 mg E_2 [11, 12, 13]. If one looked at the 24h-serum levels of each of the estrogens

following the oral administration of 2, 4, and 8 mg E_2, it became obvious that at all three doses, E_2 concentrations had already reached pretreatment values, whereas both free and total E_1 concentrations were well above the pretreatment values and showed a clear dose dependence [11]. For the repeated oral administration of E_2, it can therefore be concluded that although there will be very likely no accumulation of E_2 in the serum, for free and total E_1 an accumulation can be expected. This would as a further consequence shift the E_1/E_2-concentration ratio even more in favour of E_1 as compared to a single oral dose. Although E_1 has a lower estrogenic potency than E_2, free E_1 and total estrone form a large pool which, due to the enterohepatic recirculation of estrone sulfate and the intracellular conversion of E_1 into E_2 in target tissues, contributes substantially to the total estrogen-related pharmacodynamic response [3, 11, 14, 15].

Further points of interest are the magnitude and origin of the variability of pharmacokinetic parameters derived from the estrogen-concentration-time curves in the women. Specific information on the variability of pharmacokinetic parameters is difficult to obtain, since sometimes only mean plasma levels, or ranges are reported. Following the daily administration of 2 mg E_2 to 17 postmenopausal women over a period of two weeks, coefficients of interindividual variation (C. V.) for the AUC, calculated for free E_1 and E_2 on the last 3 treatment days, were about 50 % and 40 %, respectively [12]. During a 6 months treatment period with daily oral administration of 2–4 mg E_2, E_1 (free)- and E_2 levels in the plasma of 19 menopausal women were determined, and the interindividual variation was found to be about 60 to 70 % for both hormones. A higher value of 111 % was calculated for E_1 during the first two months of treatment [16].

Our own results obtained following single dose administration of E_2 also indicated a large interindividual variation in the pharmacokinetic parameters determined, and this may be due to several reasons. These are for example, interindividual differences in the absorption of the drug and differences in both the pattern and the activity of metabolizing enzymes in the gut wall and the liver, which are governed by environmental as well as genetic influences. Other contributing factors are the quality of the analytical method used and the number and quality of data points which were used to derive the AUC-values in the different subjects. As far as assay quality is concerned, both E_1 and E_2 assays were of adequate sensitivity and accuracy. Interassay variation on the other hand, appeared fairly high with C.V. values in the range of 15 % to 28 %. It should be noted however, that different batches of quality control samples were used during the present study, a fact that certainly contributed unfavourably to the total interassay precision. In terms of how many data points were used to calculate the AUC(0–48 h) values of E_2, there was a range of 4 to 11 concentration values which were above the detection limit following an oral dose of 4 mg, which formed the basis for the subsequent calculation. Thus, the AUC-values were documented for each individual on a different qualitative basis. The same applies to the AUC-values derived from the parenteral administration of E_2, where between 8 to 13 data points formed the basis of calculation. In conclusion, it can be stated that the large interindividual variation in the oral bioavailability of E_2 is a composite of environmental and physiological differences between individuals on the one hand and, although of less importance, methodological shortcomings on the other hand.

When the three oral administrations were compared in terms of interindividual variation of AUC-values of E_2 and free and total E_1, one observed C.V. values in the range of 70 % to 130 % for E_2, values between 70 % to 80 % for free E_1, and values in the range of 20 % to 50 % for total E_1. The variation of E_2 was somewhat higher in this study than in others, whereas the variation of free E_1 was similar to the values reported by others [12, 16]. It should be noted, however, that different from the two studies quoted, our data were obtained after single dose administration, where a higher variability is more likely to occur than during long-term treatment.

How can the issue of intraindividual variability be approached? Assuming a linear and proportional relation over the dose range of 2 to 4 mg, between dose of E_2 administered and AUC of E_2 as well as free and total E_1 measured, the intraindividual variability of the parameter AUC can be estimated from the mean of two values, normalized for a dose of 4 mg, for each subject. The mean coefficients of variations obtained were 56 ± 32 %, 33 ± 13 % and 16 ± 12 %, for E_2, free E_1 and total E_1, respectively. This indicates that over the dose range of 2 to 4 mg E_2, there was little intraindividual variation in the extent of absorption of E_2, however, a larger variation in the systemic availability of the parent compound. The corresponding value for interindividual variability of absolute bioavailability of E_2, following an oral dose of 4 mg was 95 %, and thus about twofold the estimated intraindividual variance.

In conclusion, the present study revealed an oral bioavailability of E_2 of about 5 %, with a range of 0.1 to 12 %, and a dose proportional absorption of E_2 over a dose range of 2 to 4 mg. At the higher dose of 8 mg, absorption of E_2 from the preparation used was incomplete. Pharmacokinetic parameters, like AUC, derived from serum level-time curves of E_2, free and total E_1 showed a high intra- and interindividual variability. In this respect, AUC values calculated for total E_1, proved to be less variable than those calculated for E_2 and free E_1. In the light of the very low bioavailability of oral E_2 on the one hand and the high inter- and intraindividual variability of estrogen levels in the serum on the other hand, E_2 seems not to be a likely alternative to ethinylestradiol as the estrogenic component in a combination oral contraceptive.

5. References

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