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Pharmacokinetic Studies of Estradiol Enantate in Menopausic Women

By J. C. M. Wiemeyer[1], M. Fernandez[1], J. A. Moguilevsky[2], and C. L. Sagasta[1]

Summary: *Healthy menopausic women received 10 mg i.m. of estradiol enantate (estra-1,3,5(10)-triene-3-ol-17β-heptanoate, one of the components of Perlutal® or Topasel®). Their estradiol serum levels were determined by radioimmunoassay (RIA) on days 1, 2, 3, 6, 8, 10, 13, 15, 17, 20, 22, 24, 27, 29 and 31 after the injection. Based on these data, the elimination rate constant ($K = 0.12445\ d^{-1}$), the elimination half-life ($t_{1/2} = 5.57$ d), the absorption rate constant ($K_{abs.} = 1.5050\ d^{-1}$), the absorption half-life ($t_{1/2\ abs.} = 0.46$ d) and the volume of distribution ($V_d = 5087$ l) were calculated.*
These results are compared with others not obtained by RIA and discussed from a therapeutic-clinical point of view.

Zusammenfassung: *Pharmakokinetische Untersuchungen mit Estradiolenantat bei klimakterischen Frauen*

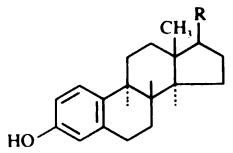
Gesunde, klimakterische Frauen wurden mit 10 mg i.m. Estradiolenantat (Estra-1,3,5(10)-trien-3-ol-17β-heptanoat, einem der Bestandteile von Perlutal® bzw. Topasel®) behandelt. An den Tagen 1, 2, 3, 6, 8, 10, 13, 15, 17, 20, 22, 24, 27, 29 und 31 nach Injektion wurden ihre Estradiol-Serumspiegel radioimmunologisch (RIA) bestimmt. Aufgrund der so bestimmten Werte wurden die Eliminationsgeschwindigkeitskonstante ($K = 0{,}12445\ d^{-1}$), die Eliminationshalbwertzeit ($t_{1/2} = 5{,}57$ d), die Absorptionsgeschwindigkeitskonstante ($K_{abs.} = 1{,}5050\ d^{-1}$), die Absorptionshalbwertzeit ($t_{1/2abs.} = 0.46$ d) und das Verteilungsvolumen ($V_d = 5087$ l) dieses Estrogen-Derivats errechnet.
Diese Resultate wurden mit anderen, nicht mittels RIA erhaltenen Ergebnissen verglichen und unter klinisch-therapeutischen Gesichtspunkten diskutiert.

Key words: *Contraceptives, injectable · Estradiol, serum levels · Estradiol enantate, clinical pharmacokinetics · Perlutal® · Topasel®*

1. Introduction

One of the most widely used injectable contraceptives in Latin America and Spain is composed by dihydroxyprogesterone acetophenide 150 mg + estradiol enantate 10 mg, monthly administered by intramuscular route*).

Estradiol enantate is a synthetic estrogen first studied by Junkmann [1] and Gauthier et al. [2]. Chemically it is estra-1,3,5(10)-triene-3-ol-17β-heptanoate, and it is represented by the following structural formula:



R = OH — estradiol (mol. wt. 272)
R = O–CO–$(CH_2)_5$–CH_3 — estradiol · enantate (mol. wt. 384)

Similar to other estrogenic esters, an estradiol enantate i.m. injection produces in animals and human beings long-term effects due to a slow saponification in the organism which releases estradiol in a progressive way.

Gual et al. [3] have performed metabolic studies on this ester in fertile women, who had received tritium-labelled estradiol enantate. Among the corresponding metabolites, estradiol, estrone and methoxyestrone were identified. They are eliminated through the kidneys, specially conjugated with glucuronic acid. Elimination half-life from plasma was estimated for estradiol enantate and their metabolic transformation products in 7.5 days.

We believe that, in a case like this, radioimmunologic techniques are particularly useful, since they allow to estimate selectively the serum levels reached by estradiol, which is the active part of this molecule, isolated from other metabolic products.

Therefore we decided to investigate the pharmacokinetic profile of estradiol enantate, employing radioimmunologic methods. In order to avoid the estradiol serum levels cyclic rhythms in fertile women we performed the studies in menopausic subjects.

2. Subjects and methods

Estradiol enantate was provided in form of injectable ampoules. Each one contained 10 mg of this substance diluted in 1 ml of benzilic benzoate + sunflower oil.

Three healthy women, 49, 48 and 51 years of age, having postmenopausic amenorrhea for five or more years, who had not received any medication during the previous month and did not present any contraindication referring to the use of estrogens, participated voluntarily in this trial. During the trial they did not receive any medication, except the investigated substance and they continued with their normal activities and usual hygiene and diets habits. Informed consent was obtained. The women at the same time took part in a study performed by Moguilevsky et al. allowing them to perform respective blood analysis [15].

Serum estradiol basal levels were determined in each subject by radioimmunoassay (RIA) at two time points: 24 h and a few minutes before the administration of a 10 mg estradiol enantate intramuscular injection. The obtained values were averaged and considered as corresponding to day 0. Estradiol serum levels were determined again on days 1, 2, 3, 6, 8, 10, 13, 15, 17, 20, 22, 24, 27, 29 and 31 after estradiol enantate administration.

The blood samples assayed were obtained between 8 and 9 a.m. of the respective days. RIA was performed according to the technique described by Abraham [4].

Results were tabulated in order to calculate the following pharmacokinetic parameters:

- elimination rate constant (K),
- elimination half-life ($t_{1/2}$),
- absorption rate constant ($K_{abs.}$),
- absorption half-life ($t_{1/2\ abs.}$)

according to Greenblatt and Koch-Weser [5, 6] and Cid Carcamo [7].

The linear regression analysis was performed using a Hewlett-Packard 86 B computer by means of a VisiCalc Plus (HP) program.

3. Results

Estradiol serum values found in each of the subjects during the study and the corresponding averages are shown in Table 1.

In Fig. 1, mean estradiol serum levels are graphically represented as a function of time. The highest peak was reached on the third day post treatment. From then on the levels decreased.

In order to calculate the elimination rate constant (K) and the elimination half-life ($t_{1/2}$) a linear regression analysis has been performed, considering the values between the 3rd and 31st days. The corresponding data are shown in Table 2 and the adjusted curve was drawn in Fig. 2, by which we estimated the following parameters:

$$K = \frac{\ln C_2 - \ln C_1}{t_2 - t_1}$$

$$K = \frac{4.32172 - 4.57061}{(24 - 22)\ \text{days}}$$

$$\boxed{K = 0.12445\ d^{-1}}$$

$$t_{1/2} = \frac{0.693}{K}$$

$$\boxed{t_{1/2} = 5.57\ d}$$

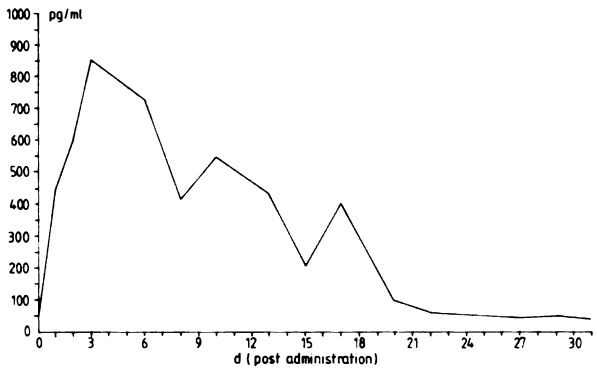


Fig. 1: Serum estradiol levels. Mean values of three subjects treated with estradiol enantate 10 mg i.m. on day 0.

Table 1: Estradiol serum levels in 3 menopausic women treated with 10 mg of estradiol enantate i.m.

Time (d) post admin.	Serum estradiol (pg/ml)			
	Subject			Mean
	1	2	3	
0	23.00	18.50	15.00	18.83
1	360.00	165.00	800.00	441.67
2	820.00	400.00	575.00	598.33
3	950.00	400.00	1200.00	850.00
6	590.00	590.00	1000.00	726.67
8	610.00	410.00	220.00	413.33
10	460.00	280.00	900.00	546.67
13	270.00	300.00	720.00	430.00
15	215.00	210.00	190.00	205.00
17	150.00	480.00	575.00	401.67
20	80.00	62.00	145.00	95.67
22	70.00	80.00	31.00	60.33
24	58.00	68.00	33.00	53.00
27	58.00	40.00	34.00	44.00
29	40.00	62.00	46.00	49.33
31	44.00	–	33.00	38.50

*) Perlutal® (manufactured by Promeco in Argentina and Mexico, by Instituto De Angeli in Brazil, by Europharma in Colombia) or Topasel® (manufactured by Europharma in Spain).

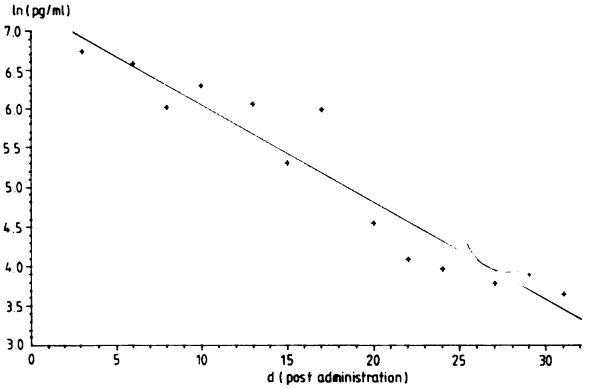


Fig. 2: Serum estradiol levels. Regression analysis. Day 3 up to day 31.

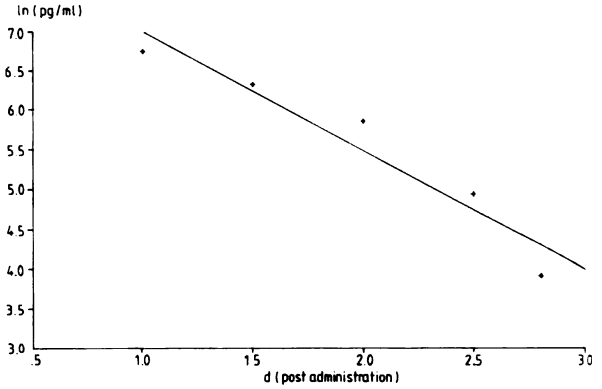


Fig. 4: Serum estradiol levels. Regression analysis. Day 0 up to day 2.8.

Table 2: Linear regression analysis of the exponential decreasing phase (elimination) of the estradiol serum levels curve as a function of time.

Time (d) post admin.	[Estradiol] mean (pg/ml)	ln [estradiol]	ln [estradiol] Regr. anal. adjust.
3	850.00	6.745	6.93506
6	726.67	6.588	6.56172
8	413.33	6.024	6.31283
10	546.67	6.304	6.06394
13	430.00	6.064	5.69061
15	205.00	5.323	5.44172
17	401.67	5.996	5.19283
20	95.67	4.561	4.81949
22	60.33	4.100	4.57061
24	53.00	3.970	4.32172
27	44.00	3.784	3.94838
29	49.33	3.899	3.69949
31	38.50	3.651	3.45060

Analysis of variance, linear regression

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Relat. F
Total	12	16.81		
Regression	1	15.31	15.31	112.63
Residual	11	1.50	0.14	

Square of R = 0.911
Y = 7.3084–0.1244 X

The absorption rate constant ($K_{abs.}$) and the absorption half-life ($t_{1/2\ abs.}$) were calculated according to the residual method [7]. With this aim, estradiol serum levels between days 0 and 3 were taken from the experimental curve. The differences between them and those theoretically determined on the extrapolated line which completes the exponential decreasing phase curve were also calculated. These differences were plotted on a semilogarithmic graph (see Fig. 3). The results of the linear regression analysis are shown in Table 3 and Fig. 4.

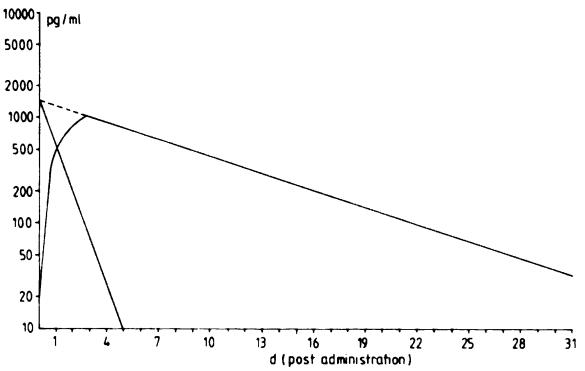


Fig. 3: Serum estradiol levels. Residual method. Day 0 up to day 3.

Table 3: Linear regression analysis of the absorption phase of the serum estradiol levels curve as a function of time (residual method).

Time (d) post admin.	A = [Estradiol] extrapolated (pg/ml)	B = [Estradiol] experimental (pg/ml)	C = A – B (pg/ml)	ln C	ln C regr. anal.
1.0	1300	440	860	6.7569	7.00403
1.5	1200	640	560	6.3279	6.25155
2.0	1150	800	350	5.8579	5.49906
2.5	1040	900	140	4.9416	4.74658
2.8	1020	970	50	3.9120	4.29508

Analysis of variance, linear regression

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Relat. F
Total	4	5.21		
Regression	1	4.83	4.83	38.08
Residual	3	0.38	0.13	

Square of R = 0.927
Y = 8.5090–1.505 X

The slope obtained represents the absorption rate constant, therefore:

$$K_{abs.} = \frac{\ln C_2 - \ln C_1}{t_2 - t_1}$$

$$K_{abs.} = \frac{5.49906 - 7.00403}{(2-1)\ \text{days}}$$

$$\boxed{K_{abs.} = 1.5050\ d^{-1}}$$

Then, the absorption half-life is:

$$t_{1/2\ abs.} = \frac{0.693}{K_{abs.}}$$

$$\boxed{t_{1/2\ abs.} = 0.46\ d}$$

4. Discussion

Based on our results, we attribute to estradiol enantate a biological half-life of 5.57 days. Gual et al. [3] estimated it to be 7.5 days. The explanation for the difference between their results and ours must be searched not only in the inter- or intraindividual variability [8] but also in the methodology employed. Gual et al. used tritium-labelled estradiol enantate and measured the total blood radioactivity; so, they did not record solely the estradiol serum levels but the whole amount of circulating metabolites produced by this substance, the pharmacokinetics of which are not necessarily identical. By means of radioimmunoassay we have studied exclusively the released estradiol levels, caused by the metabolic cleavage of the employed ester.

Because of the specificity of this method, we consider that our results refer selectively to the active metabolite, which is, no doubt, mainly responsible of the pharmacological effects of the studied substance and the most critical from a pharmaceutical point of view.

Estradiol enantate constants and the elimination and absorption half-lives correspond to a depot preparation model. When, as in this case, drugs are injected in an oily vehicle the absorption tends to be constant and slow. The oil medium forms a corpuscle between muscle and connective tissues offering a small absorption surface. In addition, not hydrosoluble but liposoluble substances are slowly delivered from the oil, thus producing a sustained release [9]. This is what happens, certainly, with estradiol enantate.

If we assume that the administered dose is totally absorbed and we calculate the apparent distribution volume (V_d), we find characteristical values of drugs which bind to the tissues or have a deep compartment distribution. Taking into account the enanthic acid molecular weight, the administered dose of estradiol enantate 10 mg corresponds to estradiol 7 mg; then, employing the following mathematical relations:

$$V_d = \frac{Q}{C}$$

$$Q = \frac{F \; D \; K_{abs.}}{K_{abs.} - K} \left(e^{-K \cdot t} - e^{-K_{abs.} t}\right)$$

$$C = C_0 \, e^{-K \cdot t} - C_0 \, e^{-K_{abs.} t}$$

where V_d = apparent distribution volume, Q = amount of drug in body, C = plasma drug concentration at time t, C_0 = extrapolated concentration at t = 0, F = absorbed drug fraction, D = administered dose, K = elimination rate constant, $K_{abs.}$ = absorption rate constant, t = time post administration, in our case, calculating C_0 = 1500 pg/ml according to Fig. 3, considering F = 1 and taking t = 13 days, there results:

V_d = 5087 l

Even though in this case it was not possible to verify F by comparing estradiol serum levels as a function of time after estradiol enantate intravenous and intramuscular administrations, such an elevated volume, which is so much higher than the one normally found in the organism, is in agreement with a wide distribution or with drug retention in a no plasmatic space, for example the injection site or the adipose tissue [10], wherefrom it is released later in a progressive way.

From a practical point of view, taking into account that estradiol enantate habitual dose in women is 10 mg i.m. once a month [11–14] and accepting that drug elimination after approximately four half-lives is almost completed [10], the risk of developing accumulation in such a therapeutic scheme seems to be very unlikely. On the contrary, this scheme is compatible with pharmacological effects for about 22 days, with a uterine bleeding because of the hormonal deprivation thereafter and with no more action until another dose is injected. If this is administered on the 7th day of a normal cycle its effect would be finished with the next menstrual bleeding. The chronic treatment, according to observations made by Gual et al. [3], is not related with changes in pharmacokinetic parameters.

5. References

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