ORIGINAL RESEARCH ARTICLE

ELSEVIER

Pharmacokinetics of Ethinyloestradiol in Rabbits After Intravenous Administration

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The pharmacokinetics of ethinyloestradiol (EE_2) after intravenous administration of 30, 50 and 100 $\mu g.kg^{-1}$ was investigated in rabbits. A high-performance liquid chromatographic (HPLC) method with electrochemical (EC) detection was used to measure EE_2 in plasma samples in order to avoid the interferences of natural oestrogens.

After compartmental analysis, the disposition of EE_2 was well described by a two-compartmental open model with mean values of: $\alpha = 0.3448 \pm 0.2922$, 0.1965 ± 0.1755, $0.3058 \pm 0.1225\ min^{-1}$, and $\beta = 0.0137 \pm 0.0018$, 0.0140 ± 0.0065, $0.0198 \pm 0.0066\ min^{-1}$, for the three doses studied, respectively.

There were no dose-related differences (ANOVA, $P < 0.05$) in a, b or V_{ss}, but significant differences were detected in clearance (90.9 ± 18.7; 80.6 ± 17.6; $116.3 \pm 21.5\ ml.min^{-1}.kg^{-1}$) between the 100 $\mu g.kg^{-1}$ group and lower dose groups. The AUC increased significantly with the doses (341.7 ± 67.1; 645.8 ± 143.9; $892.2 \pm 211.9\ ng.min.ml^{-1}$). After non-compartmental analysis there were no significant differences in λ, MRT or V_{ss} as a function of dose, but these differences were significant when Cl or AUC were compared. There were no significant differences in AUC or Cl values obtained by compartmental and non-compartmental analysis. CONTRACEPTION 1996; 53:307–312

KEY WORDS: ethinyloestradiol, pharmacokinetics, intravenous administration, rabbits, oestrogens

Introduction

Ethinyloestradiol is a synthetic oestrogen widely used as the oestrogenic component in oral contraceptives, in the treatment of menopausal symptoms, functional uterine bleeding, for the inhibition of lactation and also for palliative treatment of breast cancer in postmenopausal women and prostate cancer.[1,2] Its usefulness appears to result from its slow elimination relative to 17β-oestradiol.

Goldzieher indicated in 1994[3] that, although the pharmacokinetics of EE_2 has been studied intensively for more than a decade. Very seldom, however, has there been a complete pharmacokinetic analysis of the data. Moreover, there appears to be a wide variation in the values for some of the pharmacokinetic parameters as well as no explanation of how these parameters were calculated. Methodologic problems limit the reliability of pharmacokinetic analyses and additionally, investigators persist on using conventional contraceptive doses (30 to 35 μg) for their pharmacokinetic studies, thus straining the limits of detectability of the available analytical methods.[3]

Radioimmunoassay (RIA) is the most widely used method to measure very low levels of EE_2, but it shows cross-reactivity with several natural oestrogens[4,5] and the concurrent administration of 19-norprogestins has also raised considerable problems of specificity when they have a 17α-ethinyl side chain. On the other hand, blank values may be within the range of EE_2 levels in the later stages of the elimination phases.[3] Goldzieher also indicates that non-specific plasma blanks create a problem that is often not adequately solved; substraction of an average blank derived from a plasma pool is unreliable due to variation of the blank from cycle day.[6]

In order to solve these problems, a HPLC method with EC detection has been developed to determine EE_2 in rabbit plasma.[7] The procedure can detect as little as 50 $pg.ml^{-1}$ and it is highly reproducible.

The purpose of the present study was to establish the compartmental and non-compartmental pharmacokinetic parameters for EE_2 in rabbits after intravenous administration and determine whether or not they are dose-dependent.

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Materials and Methods

Study Design:

Experiments were carried out on 18 healthy female New Zealand white rabbits with a body weight range of 2.5–3.1 kg. Rabbits were housed in individual cages with environmentally controlled conditions (temperature was maintained at 19 ± 2°C and 12 h light–12 h dark cycle) for at least 1 week before use. These animals had free access to standard laboratory chow and water.

The rabbits were surgically fitted with a sampling cannula in the left carotid artery using Silastic® medical-grade tubing (1.02 mm ID × 2.16 mm OD) under anaesthesia with sodium pentobarbital (Barcia, Madrid, Spain), 30 $mg.kg^{-1}$, i.v. These cannulae were placed before the trial started.

The rabbits were randomly divided into three groups which received 30, 50 and 100 $\mu g.kg^{-1}$ of EE_2 (Sigma, St. Louis, MO, USA), respectively. EE_2 was administered intravenously as a solution in a mixture of saline:ethanol (4:1, v/v) into the marginal ear vein. Heparinized blood samples (3 ml) were collected from the left carotid artery before and at 3, 5, 10, 15, 20, 25, 30, 60, 90, 120, 150, 180, 210, 240, 360, 480, 720 and 1440 minutes after EE_2 administration. Plasma was immediately separated and frozen at −20°C until analysed. EE_2 in plasma was quantitated by HPLC-EC detection. The method is highly specific for EE_2 without cross-reactivity to other oestrogens. Neither heparin nor pentobarbital had any effect on the assay.

Pharmacokinetic Studies

Pharmacokinetic analysis was performed based on a compartmental as well as on a non-compartmental description of the data observed. The pharmacokinetic model best describing the plasma concentration-time courses of EE_2 was determined using the PCNONLIN computer program (Statistical Consultants, Lexington, KY)[8] with reciprocal concentration weights (1/C). Initial estimates of the parameters were determined by JANA.[9]

The best pharmacokinetic model (one, two and three compartments) was determined by application of Akaike's information criterion[10] and graphical analysis of weighted residuals. A two-compartment open model was selected and the equation used to describe EE_2 pharmacokinetics was:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where α and β are the distribution and elimination rate constants, and A and B are their respective zero time intercepts. The other compartmental parameters were calculated by standard methods.[11]

The model independent pharmacokinetic parameters were calculated using expressions based on statistical moments theory[12] and on formulae described by Gibaldi and Perrier.[11] The plasma elimination rate constant (λ) was calculated by least squares regression of the logarithm of plasma concentration versus time curve over the terminal elimination phase.

The area under the plasma concentration-time curve from time zero to the last determined sample time (AUC_{0-t}) was calculated by the trapezoidal rule, and the total area under the plasma concentration-time curve (AUC) by adding AUC_{0-t} to the residual area $AUC_{t-\infty}$ (calculated from C_t, the last experimental plasma concentration, divided by the terminal slope, λ). The area under the first moment curve from time zero to time infinity (AUMC) was calculated using the linear trapezoidal rule with extrapolation to infinity. The mean residence time (MRT) was determined by:

$$MRT = AUMC/AUC$$

The total body clearance (Cl) was calculated by dividing the dose (D) by AUC. The terminal volume of distribution (V_a) was calculated from the ratio of the total body clearance (Cl) and the terminal slope (β). The volume of distribution at steady state (V_{ss}) was determined by the equation:

$$V_{ss} = MRT \times Dose/AUC$$

Statistical Evaluation

All pharmacokinetic parameters were calculated for each animal and the data presented as arithmetic mean ± standard deviation (mean ± SD). The data obtained from the three treatments were compared for statistical significance by using the one-way and two-way analysis of variance (ANOVA). When the results were significant, the Duncan test was used to evaluate differences between data sets and a $P \leq 0.05$ was taken as the level of significance for all analyses.

Results

Mean and individual plasma concentrations of EE_2 as a function of time following intravenous administration of 30, 50 and 100 $\mu g.kg^{-1}$ to rabbits are shown in Figures 1, 2 and 3, respectively. They show that plasma EE_2 concentrations after each dose declined rapidly in a biexponential fashion with a rapid early α phase (ranging from 0.1965 to 0.3448 min^{-1}) and a terminal β phase 10-fold lower (ranging from 0.0137 to 0.0198 min^{-1}).

The pharmacokinetic parameters obtained by the compartmental analysis of these concentration-time data are summarized in Table 1. The AUC of EE_2

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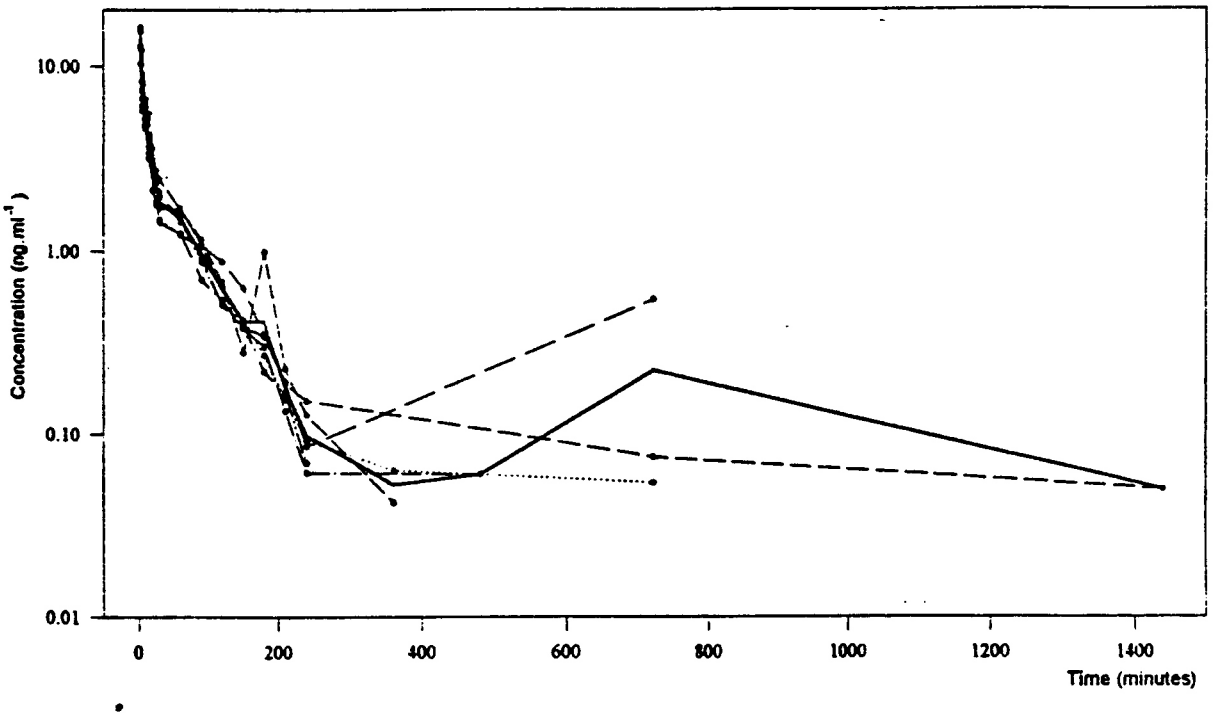

Figure 1. Individual and mean (—) plasma concentrations of EE_2 in rabbits after intravenous administration of 30 $\mu g.kg^{-1}$.

increased significantly with dose. The clearance values were similar (90.9, 80.6 and 116.3 $ml.min^{-1}.kg^{-1}$) but significant differences were detected in this parameter between the 100 $\mu g.kg^{-1}$ i.v. group and lower dose groups. The steady-state volume of distribution ranged from 4.4 to 7.2 $l.kg^{-1}$. No significant differences were found when the compartmental parameters α, β and V_{ss} were compared.

The pharmacokinetic parameters derived from non-compartmental analysis are shown in Table 2. There were no significant differences in λ, MRT, V_{ss} or V_a for the three doses studied. The AUC of EE_2 increased with dose, while Cl values were found to be statistically different between the 50 and 100 $\mu g.kg^{-1}$ groups. Finally, there were significant differences between the values obtained using the compartmental and

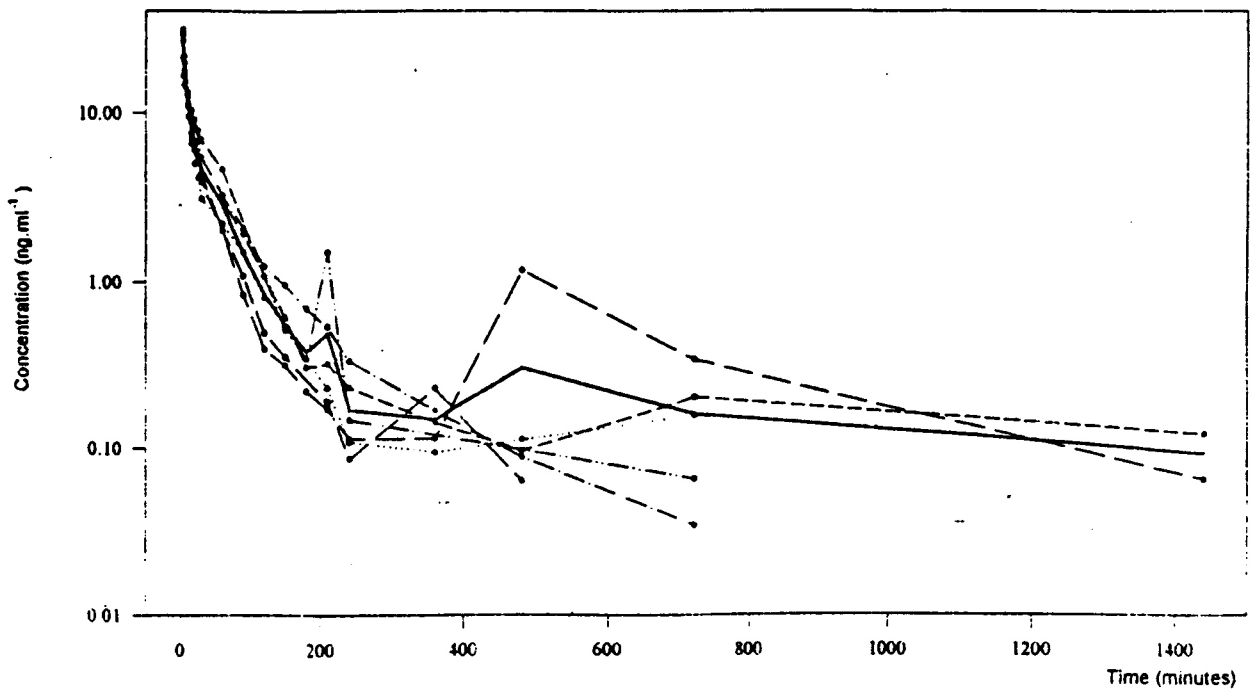

Figure 2. Individual and mean (—) plasma concentrations of EE_2 in rabbits after intravenous administration of 50 $\mu g.kg^{-1}$.

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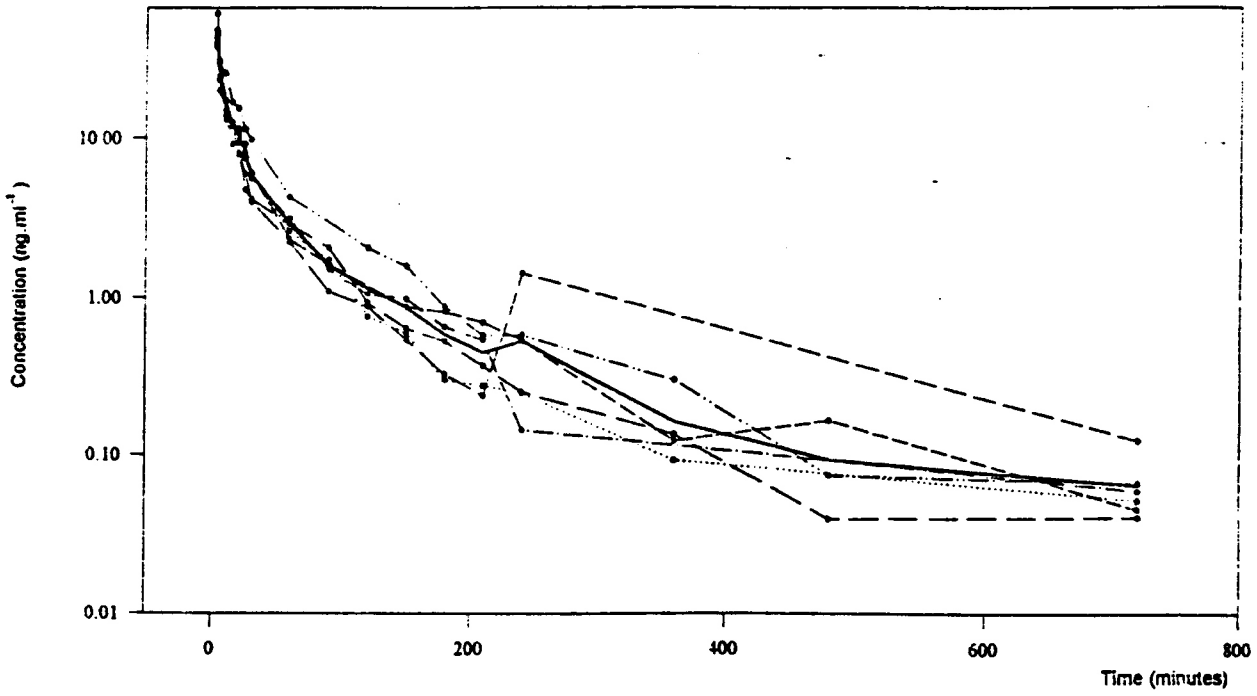

Figure 3. Individual and mean (—) plasma concentrations of EE_2 in rabbits after intravenous administration of 100 $\mu g.kg^{-1}$.

non-compartmental analysis when λ/β, V_{ss} and V_a were compared while, with the same analysis, AUC and Cl showed no significant statistical changes.

Discussion

The model used by other authors to describe EE_2 pharmacokinetics is, in practically all cases, a two-compartmental open model,[3,13–15] as in this study. Biphasic decline of EE_2 following i.v. administration with the later occurrence of a secondary peak due to enterohepatic circulation, has been previously described in several animal species[14] as well as in women.[16–18] This latter situation is not clear in the present study, in spite of the presence of secondary

Table 1. Pharmacokinetic parameters obtained by compartmental analysis in rabbits after intravenous administration of ethinyloestradiol[a]

Parameters	Dose ($\mu g.kg^{-1}$)		
	30	50	100
A ($ng.ml^{-1}$)[b]	43.36 ± 33.93	37.45 ± 28.97	78.47 ± 25.72
B ($ng.ml^{-1}$)[d,e]	3.22 ± 0.87	6.63 ± 4.26	12.28 ± 5.51
C_0 ($ng.ml^{-1}$)[b]	46.58 ± 34.63	44.08 ± 32.31	90.75 ± 30.09
α (min^{-1})[b]	0.3448 ± 0.2922	0.1965 ± 0.1755	0.3058 ± 0.1225
β (min^{-1})[b]	0.0137 ± 0.0018	0.0140 ± 0.0065	0.0198 ± 0.0066
$t_{1/2\alpha}$ (min)[b]	3.28 ± 1.81	5.34 ± 2.99	2.92 ± 2.11
$t_{1/2\beta}$ (min)[b]	51.09 ± 5.94	116.78 ± 181.78	39.95 ± 18.72
k_{12} (min^{-1})[b]	0.1968 ± 0.1866	0.1044 ± 0.1238	0.1620 ± 0.0731
k_{21} (min^{-1})[b]	0.0379 ± 0.0054	0.0417 ± 0.0266	0.0590 ± 0.0245
k_{10} (min^{-1})[b]	0.1239 ± 0.1030	0.0645 ± 0.0337	0.1045 ± 0.0384
$t_{1/2k_{10}}$ (min)[b]	8.96 ± 4.88	12.67 ± 4.58	7.54 ± 3.17
AUC ($ng.min.ml^{-1}$)[c,d,e]	341.65 ± 67.14	645.76 ± 143.87	892.17 ± 211.93
Cl ($ml.min^{-1}.kg^{-1}$)[d,e]	90.86 ± 18.71	80.64 ± 17.60	116.29 ± 21.52
V_c ($l.kg^{-1}$)[b]	1.25 ± 0.80	1.52 ± 0.71	1.24 ± 0.51
V_p ($l.kg^{-1}$)[b]	3.51 ± 0.80	5.68 ± 8.54	3.20 ± 0.94
V_{ss} ($l.kg^{-1}$)[b]	4.76 ± 1.48	7.21 ± 8.80	4.44 ± 1.40
V_a ($l.kg^{-1}$)[b]	6.78 ± 1.98	13.76 ± 21.63	6.61 ± 3.08

[a]Values are the mean ± standard deviation for six rabbits. One-way ANOVA results; [b]no statistically significant differences; significant differences (Duncan test $P \leq 0.05$) [c]between 30 and 50 $\mu g.kg^{-1}$, [d]30 and 100 $\mu g.kg^{-1}$, [e]50 and 100 $\mu g.kg^{-1}$.

Table 2. Pharmacokinetic parameters obtained by non-compartmental analysis in rabbits after intravenous administration of ethinyloestradiol[a]

Parameters	Dose ($\mu g.kg^{-1}$)		
	30	50	100
λ (min^{-1})[b,g,h]	0.0085 ± 0.0048	0.0046 ± 0.0026	0.0062 ± 0.0014
AUC ($ng.min.ml^{-1}$)[c,d,f,i,j]	331.97 ± 71.98	746.09 ± 204.41	910.02 ± 208.77
MRT (min)[b]	138.55 ± 91.82	200.21 ± 137.52	94.16 ± 31.63
Cl ($ml.min^{-1}.kg^{-1}$)[e,f,i,k]	93.80 ± 19.93	71.48 ± 19.98	114.31 ± 23.37
V_{ss} ($l.kg^{-1}$)[b,g,h]	12.13 ± 5.70	12.67 ± 6.04	10.48 ± 2.68
V_a ($l.kg^{-1}$)[b,g,h]	15.20 ± 10.10	20.93 ± 12.12	19.08 ± 4.62

[a]Values are the mean ± standard deviation for six rabbits. One-way ANOVA results: [b]no statistically significant differences; significant differences Duncan test $P \leq 0.05$: [c]between 30 and 50 $ug.kg^{-1}$; [d]30 and 100 $ug.kg^{-1}$; [e]50 and 100 $ug.kg^{-1}$. Two-way ANOVA results: [f]no statistically significant differences with compartmental parameter; [g]statistically significant differences with compartmental parameter; [h]no statistically significant differences with dose, statistically significant differences between: [i]30 and 50 $\mu g.kg^{-1}$ doses; [j]30 and 100 $ug.kg^{-1}$ doses; [k]50 and 100 $ug.kg^{-1}$ doses.

peaks in the mean plasma curves (Figures 1, 2 and 3). In the 30 and 50 $\mu g.kg^{-1}$ dose curves, these peaks are caused by two animals, while in the 100 $\mu g.kg^{-1}$ dose curve, the peak is produced by one animal. As no plasma peaks are present in 13 of the 18 animals, it is clear that a possible enterohepatic circulation would have a limited quantitative importance in the pharmacokinetics of EE_2 after i.v. administration in rabbits.

The high values obtained for the different volumes of distribution indicate a large distribution of the drug. The significant differences found in clearance do not allow us to say that pharmacokinetics of EE_2 is linear over the 30–100 $\mu g.kg^{-1}$ dose range. However, other parameters were found to be constant and, in addition, the manner of clearance variation (decreasing when dose increased from 30 to 50 $\mu g.kg^{-1}$ and afterwards increasing when dose increased from 50 to 100 $\mu g.kg^{-1}$) suggests that there may be a problem of interindividual variation rather than of dose-related differences in the parameters. The $t_{1/2\alpha}$ and $t_{1/2\beta}$ values of EE_2 obtained in the present study were lower than those previously reported in rabbits after a 100 $\mu g.kg^{-1}$ dose (14.4 and 103.8 min;[14] 30 and 180 min[15]). The Cl obtained in this study for the 100 $\mu g.kg^{-1}$ EE_2 group (116.3 $ml.min^{-1}.kg^{-1}$) is higher than the values reported by Back et al.[14] (28.3 $ml.min^{-1}.kg^{-1}$) and Dusterberg et al.[15] (37 $ml.min^{-1}.kg^{-1}$) in the same animal species. The central volume of distribution reported by Dusterberg et al.[15] (2.3 $l.kg^{-1}$) is comparable to that obtained in this study (1.2 $l.kg^{-1}$), but the non-compartmental terminal volume of distribution (19.1 $l.kg^{-1}$) is higher than that reported by Back et al.[14] (4.8 $l.kg^{-1}$). We think that the discrepancies found in these data are due to differences in the time of plasma sampling selected to give a basis for interpreting the pharmacokinetic behaviour of EE_2 in the three studies (Back et al.[13] with eleven sample times and last time at 480 minutes, Dusterberg et al.[15] with eight sample times and last time at 600 minutes and this paper with eighteen sample times and last time at 1440 minutes).

As pointed out by Goldzieher in 1994,[3] there is a wide variation in the pharmacokinetics of EE_2 reported by the different authors probably due to the difficulties in measuring plasma EE_2 levels, and also to the limitations in sampling frequency and kinetic treatment. We have solved the problem of interferences in quantification by using a specific HPLC-EC method. The effect of the sampling times is shown above by comparing data obtained with the same kinetic treatment. Furthermore, our study proves the influence of the kinetic treatment on the parameters λ/β, V_{ss} and V_a. In our opinion, the pharmacokinetics of EE_2 after i.v. administration fits a two-compartment open model, and enterohepatic recirculation cannot be correctly quantified with the data obtained in this study. Further studies, such as using larger doses, cannulating the bile duct and so on, which would allow for this quantification, are needed.

References

1. Masterson BJ. Oral contraceptive agents: current status. Am J Surg 1988;155:619–27.
2. Godsland IF, Cook D, Wynn V. Clinical and metabolic considerations of long-term oral contraceptive use. Am J Obstet Gynecol 1992;166:1955–63.
3. Goldzieher JW. Pharmacokinetics and metabolism of ethinyl estrogens. In: Goldzieher JW, Fotherby K, eds. Pharmacology of the contraceptive steroids. New York:Raven Press, 1994:127–51.
4. Hümpel M, Nieuweboer B, Wendt H, Speck U. Investigations of pharmacokinetics of ethinylestradiol to specific consideration of a possible first-pass effect in women. Contraception 1979;19:421–32.
5. Kuhnz W, Louton T, Back DJ, Michaelis K. Radioimmunological analysis of ethinylestradiol in human serum. Arzneim-Forsch Drug Res 1993;43:16–21.
6. Dibbelt L, Knuppen R, Jutting G, Heimann S, Klipping CO, Parikka-Olexih H. Group comparison of serum ethinylestradiol, SHBG and CBG levels in 83 women

using two low-dose combination oral contraceptives for three months. Contraception 1991;43:1–21.
7. Fernández N, García JJ, Diez MJ, Terán MT, Sierra M. Rapid high-performance liquid chromatographic assay of ethynyloestradiol in rabbit plasma. J Chromatogr Biomed Appl 1993;619:143–7.
8. Metzler CM, Weiner DL. PCNONLIN user's guide, version 3.0. Statistical Consultants, Lexington, KY, 1989.
9. Dunne A. JANA: A new iterative polyexponential curve stripping program. Comput Meth Prog Biomed 1985;20: 269–75.
10. Yamaoka K, Nakagawa T, Uno T. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. J Pharmacokinet Biopharm 1978;6:165–75.
11. Gibaldi M, Perrier D. Multicompartment models. In: Pharmacokinetics. New York:Marcel Dekker, 1982:45–111.
12. Yamaoka K, Nakagawa T, Uno T. Statistical moments in pharmacokinetics. J Pharmacokinet Biopharm 1978;6:547–58.
13. Fotherby K, Akpoviroro J, Abdel-Rahman. HA et al. Pharmacokinetics of ethinyloestradiol in women fron different populations. Contraception 1981;23:487–97.
14. Back DJ, Breckeridge AM, Cross KJ, Orme ML'E, Thomas E. An antibiotic interaction with ethinyloestradiol in the rat and rabbit. J Steroid Biochem 1982;16:407–13.
15. Düsterberg B, Kühne G, Tüuber U. Half-lives in plasma and bioavailability of ethinylestradiol in laboratory animals. Arzneim-Forsch Drug Res 1986;36:1187–90.
16. Back DJ, Breckéridge AM, Crawford FE et al. An investigation of the pharmacokinetics of ethinylestradiol in women using radioimmunoassay. Contraception 1979; 20:263–73.
17. Orme ML'E, Back DJ. Factors affecting the enterohepatic circulation of oral contraceptive steroids. Am J Obstet Gynecol 1990;163:2146–52.
18. Zacur HA, Burkman RT, Kimball AW, Kwiterovich P, Bell WR. Existence of multiple peaks in plasma ethinyl estradiol and norethindrone after oral administration of a contraceptive pill. J Clin Endocrinol Metab 1992;75: 1268–72.