Original Research Article

ELSEVIER

Study of the Pharmacokinetic Interaction Between Ethinylestradiol and Amoxicillin in Rabbits

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Several antibiotics have been implicated in oral contraception failure when they are administered at the same time as the oral contraceptive (OC) pill. In the present paper, a study about amoxicillin-ethinylestradiol (EE_2) pharmacokinetic potential interaction was studied. Two rabbit groups were utilized, the first group received amoxicillin (10 mg/kg) and EE_2 (30, 50 and 100 µg/kg, respectively), both by intravenous (i.v.) route. The second group received amoxicillin (oral route, 10 mg/kg/day) and EE_2 (i.v. route, 100 µ/kg) on day 1,4 and 8 of antibiotic treatment, respectively. After compartmental (two-compartment open model) and non-compartmental analysis of plasma concentrations, the statistical study (ANOVA $p \leq 0.05$) revealed that the presence of amoxicillin did not modify the EE_2 distribution and elimination pharmacokinetic parameters (by comparison with those obtained in a previous study where EE_2 was administered alone). There also were no significant differences with the time of amoxicillin oral treatment. CONTRACEPTION 1997;55:47–52

KEY WORDS: ethinylestradiol, amoxicillin, pharmacokinetic interaction, rabbits, oestrogens

Introduction

Ethinylestradiol (EE_2) is widely used as a component of the oral contraceptive (OC) pill. In order to avoid its adverse effects, it is used at low doses (30–50 µg), and if other drugs are administered at the same time and interactions appear, blood concentrations may be ineffective.

In this way, the first clinical interaction was noted in 1971 by Reimers and Jezek;[1] an increase of abnormal vaginal bleeding in OC users receiving rifampicin and other antituberculous drugs. Since then, antibiotics and chemotherapeutic agents have frequently been implicated in contraception failure. There have been numerous case reports of OC users becoming pregnant while receiving ampicillin, tetracycline, chloramphenicol, griseofulvin, sulfamides and others.[2–8]

The postulated mechanism by which broad-spectrum antibiotics interfere with OCs is alteration of gut flora so that less estrogen is reabsorbed.[9] There is good evidence that EE_2 can be conjugated with both sulfuric and glucuronic acids, after which it undergoes enterohepatic circulation.[10,11] These conjugates are excreted in the bile and thus reach the gut, where they may be hydrolyzed to liberate unchanged EE_2 which can be reabsorbed into the portal circulation.

Oral antibiotics kill off the bacteria responsible for the hydrolytic process, so these drugs can modify the pharmacokinetics of EE_2. Thus, any conjugates of this estrogen present in the lower part of the gastrointestinal tract are lost in the feces since they are too hydrophilic to be absorbed themselves, and potentially the plasma concentration of EE_2 would fall. Back et al.[12,13] demonstrated in rats and rabbits that ampicillin and other antibiotics cause a fall in the plasma concentration of EE_2 by interfering with the enterohepatic recirculation of this estrogen.

Clinical pharmacokinetic studies in women have been unsuccessful in demonstrating any consistent effect of antibiotics on plasma concentrations of contraceptive steroids,[14–18] although they have been implicated in pill failure. The only pharmacokinetic study apparently showing a decrease in EE_2 AUC in the presence of an antibiotic (minocycline) was cited by Shenfield and Griffin.[19] However, this interaction is perhaps the most controversial of those in which oral contraceptive steroids are involved, and the clinical importance of this potential interaction has not been determined.[20]

The present study was planned to establish if amoxicillin modifies the EE_2 distribution and excretion pharmacokinetic parameters after intravenous administration in the rabbit. Another objective was to prove if a modification in the EE_2 enterohepatic recirculation causes these alterations.

Amoxicillin is a widely used broad-spectrum peni-

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cillin and the potential interaction with EE_2 is of considerable importance since many women taking oral contraceptive steroids receive amoxicillin treatment. Several cases of pregnant women reported to the British Committee on Safety of Medicines suggest a possible interaction,[21] but there are no studies that definitively address the question of interaction between these two drugs.

Materials And Methods

Study Design

Thirty-six New Zealand female white rabbits weighing 2.8–3.2 kg were used. During the experimental period, all animals were housed in individual metal cages which allowed the isolation of feces in a lower container to avoid coprophagy. The environmental conditions were: constant humidity (55 ± 10%), temperature (19 ± 2°C) and a 12 h light-12 h dark cycle. The animals were maintained on laboratory chow and water ad libitum.

The 36 rabbits were randomly divided into two groups of 18 animals each. All the rabbits of the first group received 10 mg/kg of amoxicillin intravenously and, at the same time, EE_2 intravenously at the following doses:

- 30 µg/kg (first subgroup, N = 6)
- 50 µg/kg (second subgroup, N = 6) and
- 100 µg/kg (third subgroup, N = 6).

Amoxicillin and EE_2 were administered as a solution in a mixture of saline:ethanol (4:1, v/v) into the marginal ear vein.

On the other hand, the 18 rabbits of the second group were orally treated with 10 mg/kg/day of amoxicillin as an aqueous solution using a gavage needle and also received intravenously 100 µg/kg of EE_2 on day 1 (first subgroup), on day 4 (second subgroup) and on day 8 (third subgroup) of antibiotic treatment.

The simultaneous administration of EE_2 (30, 50 and 100 µg/kg) and amoxicillin (10 mg/kg) by an intravenous route (first group) was carried out to prove if the distribution and excretion pharmacokinetic parameters of EE_2 were modified by the presence of the antibiotic. These parameters were obtained in a previous study[22] where EE_2 was administered alone.

In order to verify if oral treatment with amoxicillin modified the pharmacokinetic parameters of EE_2 in a different way, three rabbit subgroups were used (second group). These animals received the antibiotic by an oral route (10 mg/kg/day) and EE_2 intravenously (100 µg/kg) on day 1, 4 and 8 of antibiotic treatment. If the antibiotic modified the pharmacokinetic parameters of EE_2 observed in the former group, it would suggest a significant alteration in EE_2 enterohepatic recirculation.

The rabbits were anesthetized with sodium pentobarbital, 30 mg/kg, i.v. and the left carotid artery was canulated with Silastic medical-grade tubing 1.02 mm ID × 2.16 mm OD. Amoxicillin and EE_2 administration was carried out after total recovery from anaesthesia was achieved.

Blood samples (3 ml) were taken, via this canula, prior to each dose of EE_2 and at 3, 5, 10, 15, 20, 25, 30, 60, 90, 120, 150, 180, 210, 240, 480, 720 and 1440 minutes after dosing. The blood was taken into heparinized containers, centrifuged at 2000 rpm for 10 minutes and the supernatant plasma was removed and stored at −20°C prior to analysis. EE_2 in plasma was quantitated by HPLC with electrochemical detection.[23] Intra- and interday accuracy and precision were within 10%.

Pharmacokinetic Studies

Individual plasma EE_2 concentration-time data were analysed using both compartmental and non-compartmental methods.

Compartmental analysis: The pharmacokinetic model best describing the plasma concentration-time courses of EE_2 was determined using the PCNONLIN computer program (Statistical Consultants, Lexington, KY)[24] with reciprocal concentration weights (1/C). Initial estimates of the parameters were determined by JANA.[25] The best pharmacokinetic model (one, two and three compartments) was determined by application of Akaike's information criterion[26] 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.[27]

Non-compartmental analysis: This study was performed using the statistical moments theory[28] and according to the standard formulae.[27] 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 parameters studied included the area under the plasma concentration-time curve from time zero to the last determined sample time (AUC_{0-t}), the total area under the plasma concentration-time curve (AUC) and the area under the first moment curve from time zero to time infinity (AUMC). AUC and AUMC were used for the estima-

tion of the mean residence time (MRT), the volume of distribution at steady state (V_{ss}), the terminal volume of distribution (V_a) and the total body clearance (Cl).

Statistical Evaluation

All pharmacokinetic parameters were calculated for each animal and the results expressed as arithmetic mean ± standard deviation (mean ± SD). The estimated pharmacokinetic parameters for the two groups were compared for statistical significance by using the one-way and two-way analysis of variance (ANOVA), and the Duncan test was used to evaluate differences between data sets when the results were significant. The significance level was considered to be $p \leq 0.05$.

Results

The mean EE_2 plasma concentration time profiles for the three doses (30, 50 and 100 µg/kg) obtained after its administration with amoxicillin (10 mg/kg) by the intravenous route are shown in Figure 1. Figure 2 includes the mean plasma concentrations of EE_2 as a function of time following intravenous administration (100 µg/kg) on day 1, 4 and 8 of amoxicillin oral treatment. After compartmental analysis, the plasma concentration-time curves were best resolved in all experiments into a two-compartment open model.

EE_2 and Amoxicillin Intravenous Administration

The EE_2 pharmacokinetic parameters determined by compartmental analysis are given in Table 1. The values obtained for AUC increased significantly with dose (314.6, 556.6 and 911.5 ng · min · m^{-1}, respectively). The steady-state volume of distribution ranged from 4.2 to 10.01 · kg^{-1} and clearance values ranged from 91.6 to 110.6 ml · min^{-1} · kg^{-1}. No significant differences were found when the compartmental parameters α, β, Cl and V_{ss} were compared.

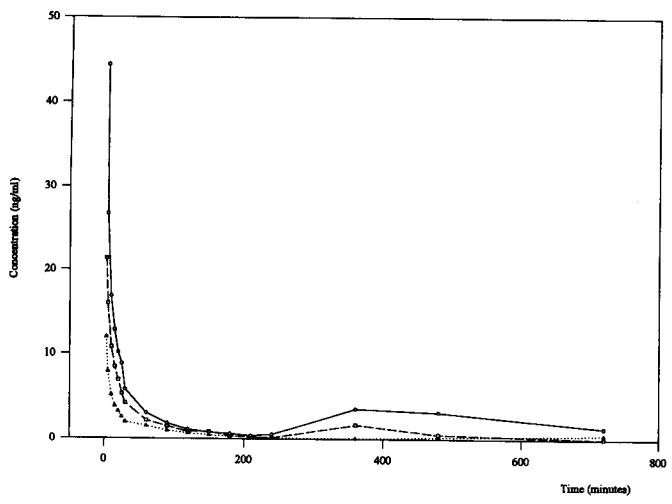

Figure 1. Mean plasma concentrations of EE (···▲··· 30 µg/kg; --□-- 50 µg/kg; –O– 100 µg/kg) in the presence of amoxicillin (i.v., 10 mg/kg) in rabbits after intravenous administration.

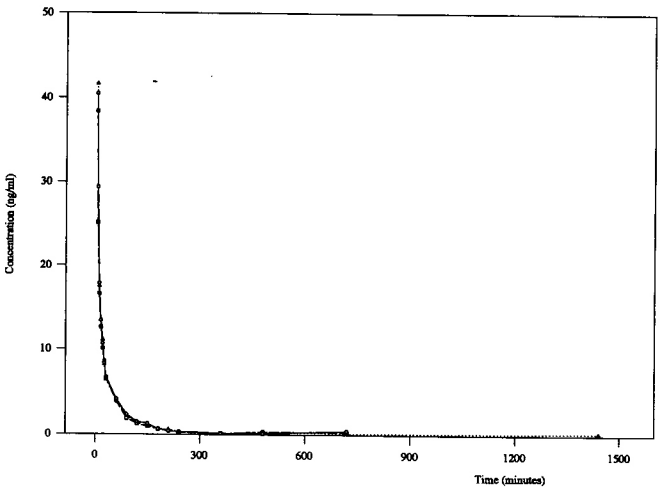

Figure 2. Mean plasma concentrations of EE_2 (100 µg/kg) in rabbits after intravenous administration in the presence of amoxicillin (oral, 10 mg/kg/day) on day 1 (···▲···), 4 (--□--) and 8 (–O–), of amoxicillin oral treatment.

The pharmacokinetic parameters obtained from non-compartmental analysis are shown in Table 2. There were no significant differences in λ, MRT, Cl, V_{ss} or V_a for the three doses studied, while AUC values were found to be statistically different.

On the other hand, there were significant differences between the values obtained for λ/β, V_{ss}, V_a, Cl and AUC when the compartmental and non-compartmental parameters were compared.

EE_2 Intravenous Administration and Amoxicillin Oral Administration

In Figure 2, the similar evolution of the three concentration-time curves obtained for EE_2 on day 1, 4 and 8 of amoxicillin treatment can be appreciated. As regards the last point (1440 minutes) present in the curve obtained on day 1 of amoxicillin treatment, it was only provided by one rabbit.

The pharmacokinetic parameters derived from compartmental analysis are summarized in Table 3. In this case, V_{ss} ranged from 4.7 to 5.0 l · kg^{-1} and Cl from 103.5 to 114.9 ml · min^{-1} · kg^{-1}. AUC values were 938.9, 894.1 and 980.4 ng · min · ml^{-1} for EE_2 administration on day 1, 4 and 8 of amoxicillin treatment, respectively. One-way ANOVA analysis revealed no significant differences in the pharmacokinetic parameters with the time of amoxicillin treatment.

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Table 1. Pharmacokinetic parameters obtained by compartmental analysis in rabbits after intravenous administration of ethinylestradiol[a] in the presence of amoxicillin (i.v.)

Parameters	Dose ($\mu g \cdot kg^{-1}$)		
	30	50	100
A ($ng \cdot ml^{-1}$)[d,e]	15.308 ± 3.662	26.600 ± 13.899	56.389 ± 21.927
B ($ng \cdot ml^{-1}$)[d]	3.339 ± 1.467	6.732 ± 2.679	9.618 ± 5.778
C_0 ($ng \cdot ml^{-1}$)[d,e]	18.647 ± 4.642	33.332 ± 15.085	66.007 ± 25.528
α (min^{-1})[b]	0.2060 ± 0.0599	0.1948 ± 0.1525	0.1903 ± 0.0930
β (min^{-1})[b]	0.0137 ± 0.0018	0.0169 ± 0.0050	0.0161 ± 0.0086
$t_{1/2\alpha}$ (min)[b]	3.608 ± 1.038	4.859 ± 2.257	4.439 ± 2.087
$t_{1/2\beta}$ (min)[b]	51.327 ± 6.263	44.201 ± 13.329	112.838 ± 180.420
k_{12} (min^{-1})[b]	0.1115 ± 0.0402	0.0971 ± 0.1021	0.0895 ± 0.0495
k_{21} (min^{-1})[b]	0.0489 ± 0.0202	0.0551 ± 0.0329	0.0433 ± 0.0276
k_{10} (min^{-1})[b]	0.0593 ± 0.0092	0.0595 ± 0.0261	0.0736 ± 0.0313
$t_{1/2k10}$ (min)[b]	11.924 ± 1.805	12.993 ± 3.855	10.614 ± 3.634
AUC ($ng \cdot min \cdot ml^{-1}$)[c,d,e]	314.585 ± 63.372	556.638 ± 89.221	911.518 ± 85.605
Cl ($ml \cdot min^{-1} \cdot kg^{-1}$)[b]	97.990 ± 15.755	91.578 ± 13.319	110.560 ± 10.944
V_c ($l \cdot kg^{-1}$)[b]	1.687 ± 0.379	1.741 ± 0.687	1.665 ± 0.483
V_p ($l \cdot kg^{-1}$)[b]	3.878 ± 0.911	2.451 ± 0.584	8.333 ± 13.628
V_{ss} ($l \cdot kg^{-1}$)[b]	5.564 ± 1.155	4.192 ± 0.942	9.997 ± 13.937
V_a ($l \cdot kg^{-1}$)[b]	7.330 ± 1.690	5.720 ± 1.411	16.612 ± 25.074

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

Table 4 includes the pharmacokinetic parameters calculated by non-compartmental analysis. The results of statistical analyses show no significant differences in the different pharmacokinetic parameters determined.

Likewise, there were no significant differences with pharmacokinetic analysis when λ/B, AUC and Cl compartmental and non-compartmental values were compared, while V_{ss} and V_a values showed significant changes.

Finally, one-way and two-way analysis of variance (ANOVA) were carried out to determine the influence of the presence of amoxicillin and the pharmacokinetic analysis on the pharmacokinetics of EE_2. These analyses included the pharmacokinetic parameters of EE_2 (100 µg/kg) calculated after its administration alone[22] and with amoxicillin (oral and i.v.). The results of these analyses showed that the presence of amoxicillin does not modify the pharmacokinetics of EE_2 in any of the cases proposed in this study.

Discussion

In this paper, amoxicillin had no significant effect on the plasma concentrations and pharmacokinetic parameters of EE_2 administered intravenously. Back et al.[13] studied the influence of several antibiotics (neomycin, lincomycin, oral route) on the pharmacokinetics of EE_2 (i.v. route) in rabbits. These authors found that the mean concentration-time curve profiles of the estrogen in the presence of antibiotics was similar to that obtained with EE_2 administered alone, but with two important differences. The first one was the displacement of the secondary peak to shorter times (from 540 to 300 minutes) or the abolishment of this peak, and the second one was a decrease in the value

Table 2. Pharmacokinetic parameters obtained by non-compartmental analysis in rabbits after intravenous administration of ethinylestradiol[a] in the presence of amoxicillin (i.v.)

Parameters	Dose ($\mu g \cdot kg^{-1}$)		
	30	50	100
λ (min^{-1})[b,e,f]	0.0085 ± 0.0033	0.0052 ± 0.0033	0.0065 ± 0.0078
AUC ($ng \cdot min \cdot ml^{-1}$)[c,d,e,g,h]	347.88 ± 64.18	739.72 ± 263.83	1426.29 ± 713.76
MRT (min)[b]	177.954 ± 102.712	185.397 ± 68.599	335.325 ± 188.638
Cl ($ml \cdot min^{-1} \cdot kg^{-1}$)[b,e,f]	88.486 ± 16.282	67.175 ± 23.528	84.265 ± 41.094
V_{ss} ($l \cdot kg^{-1}$)[b,e,f]	15.699 ± 8.521	11.454 ± 2.056	22.756 ± 7.443
V_a ($l \cdot kg^{-1}$)[b,e,f]	11.631 ± 4.306	15.393 ± 6.538	21.821 ± 10.510

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

Table 3. Pharmacokinetic parameters obtained by compartmental analysis in rabbits after intravenous administration of ethinylestradiol (100 μg/kg)[a] on day 1, 4, and 8 of amoxicillin oral treatment

Parameters	Day		
	1	4	8
A ($ng \cdot ml^{-1}$)[b]	50.302 ± 29.788	54.867 ± 28.708	57.039 ± 22.719
B ($ng \cdot ml^{-1}$)[b]	15.778 ± 10.410	11.721 ± 4.488	11.269 ± 4.945
C_0 ($ng \cdot ml^{-1}$)[b]	66.080 ± 25.764	66.588 ± 32.845	68.308 ± 26.944
α (min^{-1})[b]	0.1877 ± 0.1473	0.2315 ± 0.1447	0.2079 ± 0.1245
β (min^{-1})[b]	0.0409 ± 0.0576	0.0178 ± 0.0036	0.0158 ± 0.0041
$t_{1/2\alpha}$ (min)[b]	10.756 ± 16.836	3.951 ± 2.111	4.168 ± 1.777
$t_{1/2\beta}$ (min)[b]	34.335 ± 15.254	40.418 ± 8.778	46.470 ± 12.529
k_{12} (min^{-1})[b]	0.1088 ± 0.0859	0.1218 ± 0.0997	0.1081 ± 0.0857
k_{21} (min^{-1})[b]	0.0494 ± 0.0133	0.0550 ± 0.0226	0.0470 ± 0.0228
k_{10} (min^{-1})[b]	0.0703 ± 0.0268	0.0724 ± 0.0275	0.0686 ± 0.0210
$t_{1/2k10}$ (min)[b]	10.839 ± 3.173	10.662 ± 3.633	10.813 ± 2.846
AUC ($ng \cdot min \cdot ml^{-1}$)[b]	938.875 ± 47.651	894.088 ± 162.891	980.427 ± 128.778
Cl ($ml \cdot min^{-1} \cdot kg^{-1}$)[b]	106.736 ± 5.333	114.875 ± 20.128	103.537 ± 14.102
V_c ($l \cdot kg^{-1}$)[b]	1.671 ± 0.506	1.811 ± 0.800	1.635 ± 0.538
V_p ($l \cdot kg^{-1}$)[b]	3.002 ± 0.466	3.174 ± 0.708	3.345 ± 1.132
V_{ss} ($l \cdot kg^{-1}$)[b]	4.673 ± 0.619	4.984 ± 1.418	4.980 ± 1.560
V_a ($l \cdot kg^{-1}$)[b]	5.281 ± 2.418	6.796 ± 2.251	7.125 ± 2.849

[a]Values are the mean ± standard deviation for six rabbits. One-way ANOVA results: [b]no statistically significant differences.

of AUC. However, in the present study, the secondary peak appeared at higher times: 480 minutes on day 1 and 4, and 720 minutes on day 8 of oral amoxicillin treatment, while it appeared at 210–240 minutes when EE_2 was administered alone.[22] On the other hand, we found that the pharmacokinetic parameters of EE_2 were not significantly different when the estrogen was given alone[22] and in the presence of intravenous amoxicillin. These results indicate that the antibiotic has no effect on the distribution and elimination pharmacokinetic parameters of EE_2.

A prevailing opinion has been that the conjugates of EE_2 have relatively long half-lives and through enterohepatic recirculation might be deconjugated to provide a slow-release reservoir of EE_2[29,30] and that broad-spectrum antibiotics interfere with estrogen reabsorption.[9] However, we found no significant differences between EE_2 pharmacokinetic parameters when it was administered alone and with oral amoxicillin.

Taking into account that amoxicillin did not lower plasma EE_2 concentrations in this study, and that the direct conjugation is a minor pathway of estrogen metabolism,[31] a non-significant EE_2-amoxicillin pharmacokinetic interaction is suggested.

References

1. Reimers D, Jezek A. Rifampicin und andere Antituberkulostatika bei gleichzeitiger oraler Kontrazeption. Prax Pneumol 1971;25:255–62.
2. von Hempel E, Böhm W, Carol W, Klinger, G. Medikamentose Enzyminduktion und hormonale Kontrazeption. Zentrabl Gynäkol 1973;95:1451–7.
3. Dossetor J. Drug interactions with oral contraceptives. Br Med J 1975;4:467–8.
4. Bacon JF, Shenfield GM. Pregnancy attributable to in-

Table 4. Pharmacokinetic parameters obtained by non-compartmental analysis in rabbits after intravenous administration of ethinylestradiol (100 μg/kg)[a] on day 1, 4, and 8 of amoxicillin oral tratment

Parameters	Day		
	1	4	8
λ (min^{-1})[b,c,e]	0.0076 ± 0.0038	0.0096 ± 0.0044	0.0101 ± 0.0036
AUC ($ng \cdot min \cdot ml^{-1}$)[b,c,e]	983.77 ± 172.50	889.01 ± 138.45	919.06 ± 98.06
MRT (min)[b]	146.565 ± 135.592	119.188 ± 68.414	93.969 ± 47.323
Cl ($ml \cdot min^{-1} \cdot kg^{-1}$)[b,c,e]	103.839 ± 15.062	114.769 ± 17.700	109.848 ± 12.197
V_{ss} ($l \cdot kg^{-1}$)[b,d,e]	13.598 ± 9.171	13.682 ± 8.136	10.263 ± 4.772
V_a ($l \cdot kg^{-1}$)[b,d,e]	19.173 ± 14.909	14.286 ± 6.223	12.268 ± 5.122

[a]Values are the mean ± standard deviation for six rabbits. One-way ANOVA results: [b]no statistically significant differences. Two-way ANOVA results: [c]no statistically significant differences with compartmental parameter; [d]statistically significant differences with compartmental parameter; [e]no statistically significant differences with dose.

teraction between tetracycline and oral contraceptives. Br Med J 1980;1:293.
5. De Sano EA, Hurley SC. Possible interactions of antihistamines and antibiotics with oral contraceptive effectiveness. Fertil Steril 1982;37:853–4.
6. van Dijke CPH, Weber JCP. Interaction between oral contraceptives and griseofulvin. Br Med J 1984;288: 1125–6.
7. Bainton R. Interaction between antibiotic therapy and contraceptive medication. Oral Surg 1986;61:453–5.
8. Back DJ, Orme ML'E. Drug interactions. In: Goldzieher JW, Fotherby K, eds. Pharmacology of the contraceptive steroids. New York: Raven Press, 1994:407–25.
9. Shenfield GM. Drug interactions with oral contraceptive preparations. Med J Australia 1986;144:205–11.
10. Back DJ, Breckenridge AM, MacIver M, et al. The gut wall metabolism of ethinylestradiol and its contribution to the pre-systemic metabolism of ethinylestradiol in humans. Br J Clin Pharmacol 1982;13:325–30.
11. Rogers SM, Back DJ, Orme ML'E. Intestinal metabolism of ethinyloestradiol and paracetamol in vitro: studies using Ussing chambers. Br J Clin Pharmacol 1987;23:727–34.
12. Back DJ, Breckenridge AM, Challiner M, et al. The effect of antibiotics on the enterohepatic circulation of ethinyloestradiol and norethisterone in the rat. J Steroid Biochem 1978;9:527–31.
13. Back DJ, Breckenridge 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.
14. Friedman CI, Huneke AL, Kim MH, Powell J. The effect of ampicillin on oral contraceptive effectiveness. Obstet Gynecol 1980;55:33–7.
15. Joshi JV, Joshi UM, Sankholi GM, et al. A study of interaction of low-dose combination oral contraceptives with ampicillin and metronidazole. Contraception 1980;22:643–52.
16. Back DJ, Breckenridge AM, MacIver M, et al. The effects of ampicillin on oral contraceptive steroids in women. Br J Clin Pharmacol 1982;14:43–8.
17. Orme ML'E, Back DJ. Interactions between oral contraceptive steroids and broad spectrum antibiotics. Clin Exp Dermat 1986;11:327–31.
18. Murphy AA, Zacur HA, Charache P, Burkman RT. The effect of tetracycline on levels of oral contraceptives. Am J Obstet Gynecol 1991;164:28–33.
19. Shenfield GM, Griffin JM. Clinical pharmacokinetics of contraceptive steroids. An update. Clin Pharmacokinet 1991;20:15–37.
20. McEvoy GK, ed. A.H.F.S. Drug Information. Bethesda: American Society of Health-System Pharmacists, 1996: 2259.
21. Back DJ, Grimmer SFM, Orme ML'E, et al. Evaluation of Committee on Safety of Medicines yellow card reports on oral contraceptive drug interactions with anticonvulsants and antibiotics. Br J Clin Pharmacol 1988;25:527–32.
22. Fernández N, Sierra M, Diez MJ, Terán MT, Sahagún AM, García JJ. Pharmacokinetics of ethinyloestradiol in rabbits after intravenous administration. Contraception 1996;53:307–12.
23. Fernández N, García JJ, Diez MJ, Terán MT, Sierra M. Rapid high-performance liquid chromatographic assay of ethinyloestradiol in rabbit plasma. J Chromatogr 1993;619:143–7.
24. Metzler CM, Weiner DL. PCNONLIN user's guide, version 3.0. Lexington, KY: Statistical Consultants, 1989.
25. Dunne A. JANA: a new iterative polyexponential curve stripping program. Comput Meth Prog Biomed 1985;20: 269–75.
26. 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.
27. Gibaldi M, Perrier D. Multicompartment models. In: Pharmacokinetics. New York: Marcel Dekker, 1982: 45–111.
28. Yamaoka K, Nakagawa T, Uno T. Statistical moments in pharmacokinetics. J Pharmacokinet Biopharm 1978; 6:547–58.
29. Fotherby K. Pharmacokinetics of ethynyl estradiol in humans. Methods Find Exp Clin Pharmacol 1982;4: 133–41.
30. Stern MD. Pharmacology of conjugated estrogens. Maturitas 1982;4:333–9.
31. Goldzieher JW. Pharmacokinetics and metabolism of ethynyl estrogens. In: Goldzieher JW, Fotherby K, eds. Pharmacology of the contraceptive steroids. New York: Raven Press, 1994:127–51.