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INTRASUBJECT VARIABILITY IN THE PHARMACOKINETICS OF ETHYNYLOESTRADIOL

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Summary—Intrasubject and intersubject variability in the metabolism of ethynyloestradiol (EE) was assessed in a cross-over randomized study of 6 women who each received 3 months treatment with 50 μg EE and 50 μg EE with 250 μg levonorgestrel (LNG). Blood samples were collected at the end of each treatment month, assayed for EE and the half-life of elimination (T_{el}) and bioavailability (area under the serum concentration–time curve, AUC) calculated. Intrasubject variability for T_{el} and AUC varied markedly; the variability was random and not correlated with the formulation administered. The intrasubject variability for T_{el} and AUC was 31 and 17%, respectively, and intersubject variability 66 and 95%. The intersubject range of values was more than 3-fold for both T_{el} and AUC and the intrasubject range about 2-fold. The pharmacokinetics of EE were not influenced by LNG; mean values for T_{el} and AUC were 17.3 ± 5.5 h and 11.1 ± 3.8 ng/ml/h, respectively, when EE was administered alone compared with 16.4 ± 4.8 h and 12.5 ± 3.9 ng/ml/h when given with LNG. However, EE influenced the metabolism of LNG; T_{el} for LNG was 19.3 ± 4.2 h when administered alone and significantly higher (30.0 ± 11.2 h) when given with EE. There was no correlation between the rate of metabolism of EE and that of LNG. The intrasubject variability shown in this and other studies suggests that genetic factors are less important in intersubject variability than previously thought. Some implications of intrasubject variability are discussed.

INTRODUCTION

Large intersubject [1] and intrasubject [2] variations in the disposition of many drugs have been reported. Whereas intersubject variability in the metabolism of the contraceptive steroids has been described [3], there has until recently been no investigation of possible intrasubject variability in the pharmacokinetics of these steroids. This report describes such variations for ethynyloestradiol (EE) and complements those recently published [4] for levonorgestrel (LNG).

EXPERIMENTAL

The data presented are derived from a previous study [5] in which 6 women were dosed successively with 50 μg EE, 50 μg EE with 250 μg LNG, 250 μg LNG or with a placebo. Each treatment was administered orally daily for 3 months with blood samples taken at 2, 8 and 24 h after dosing on the last day of each treatment period. The subjects were randomized to the initial treatment and crossed-over after 3 months of use. The women were 33–53 years in age and had undergone both hysterectomy and bilateral ovariectomy. In the blood samples taken during periods of treatment containing EE, the concentration of EE was determined by RIA [6]. The approximate half-life of elimination (T_{el}) was calculated from the concentrations in the 8 and 24 h samples and the approximate bioavailability, measured by the area under the serum concentration–time curve (AUC), was calculated from the concentrations in the 2, 8 and 24 h samples.

RESULTS

Because blood sampling was performed at only 2, 8 and 24 h after administration of EE, it was necessary to determine whether calculations of T_{el} and AUC based upon these three sample times were valid. To do this, values for these two pharmacokinetic parameters, in published reports of investigations in which multiple sampling had been performed, were compared with values calculated by the present author from the published serum concentrations at 2, 8 and 24 h. These comparisons are shown in Table 1. For T_{el}, calculated values were slightly larger than reported values but there was a good correlation ($r = 0.94$) between the two sets of values.

Table 1. Comparison of reported (Rep.) and calculated (Calc.) values for T_{el} and AUC

T_{el}		AUC (ng/ml/h)		Dose		
Rep.	Calc.	Rep.	Calc.	(μg)	n	Ref.
10.0	12.0	0.91	0.50	35	30	[7]
12.6	12.8	0.75	0.68	50	8	[7]
6.4	9.6	1.16	1.28	80	10	[7]
14.7	15.8	—	—	50	5	[7]
8.1	10.8	—	—	100	10	[7]
12.7	15.3	8.2	8.4	50	83	[8]
6.9	8.0	1.06	0.96	50	18	[9]
—	—	59.2	57.1	3000	6	[10]
—	—	1.04	0.96	35	24	[11]

For details see the text, n denotes the number of subjects.

Table 4. Intra and intersubject variability in half-life of elimination (T_{el}) and bioavailability (AUC) of EE after administration of EE alone or with LNG

	T_{el}		AUC	
	EE + LNG	EE	EE + LNG	EE
Mean variation %				
Intrasubject	31.4	31.9	10.8	22.6
Intersubject	54.3	76.8	92.8	95.0
Variation ratio				
Intrasubject	1.43	1.38	1.13	1.25
Intersubject	1.77	2.17	2.81	3.22
Analysis of variance (c.v.)				
Intrasubject	7.8	12.0	4.7	9.8
Intersubject	24.4	25.0	35.1	37.8

Values for AUC depend on the dose and whether calculated for 0–24 h or to infinity. With one exception, the reason for which is not clear, there was a good correlation ($r = 0.99$) between the reported and calculated values. The former tended to be slightly larger than the latter, since the calculated values, based only upon one measurement in the early post-administration phase, would have led to a truncation of the absorption peak. These comparisons therefore demonstrate that calculation of these two pharmacokinetic parameters, determined from the serum EE concentrations at 2, 8 and 24 h, is valid. This is in agreement with similar calculations for LNG based upon more extensive data [4].

Calculated values for T_{el} and AUC for each of the subjects in each cycle of treatment are shown in Tables 2 and 3, respectively. For some study periods (e.g. subject 6, EE 50 + LNG 250 μg; subject 4, EE 50 μg) monthly variations in T_{el} were small, whereas for others (e.g. subject 2, EE 50 + LNG 250 μg; subject 5, EE 50 μg) the variation was much wider. It will also be noted that subjects may show a large variation when receiving one formulation but not when receiving the other (see subjects 3 and 6). The mean intrasubject variation % during treatment with EE + LNG (31.4%) did not differ significantly from that with EE alone (31.9%), nor was there any difference in the variation ratio. The intersubject variability was higher both in terms of variation % and variation ratio when EE was administered alone (Table 4) but these differences were not statistically significant.

Similar considerations apply to the values for bioavailability shown in Table 3. For some study periods monthly variations were small

Table 2. Half-life of elimination (T_{el}) of ethynyloestradiol (EE) in a group of 6 women during each of 3 months of treatment with EE 50 μg and LNG 250 μg or EE 50 μg alone

Formulation:	EE 50 + LNG 250 μg						EE 50 μg					
Subject:	1	2	3	4	5	6	1	2	3	4	5	6
Month 1	12.6	26.6	16.8	12.8	17.0	11.4	16.8	21.3	20.4	18.8	12.8	10.0
Month 2	18.0	12.3	14.1	10.4	22.9	11.5	19.3	32.6	13.7	—	20.4	11.9
Month 3	20.8	22.2	14.6	—	—	11.7	14.3	19.9	15.7	16.8	14.7	11.9
Mean	17.1	20.4	15.2	11.6	20.0	11.5	16.8	24.6	16.6	17.8	16.0	11.3
Variation %	47.9	70.1	17.8	20.7	29.5	2.6	29.8	45.5	40.4	11.2	47.5	16.8
Variation ratio	1.7	2.2	1.2	1.2	1.3	1.0	1.3	1.6	1.5	1.1	1.6	1.2
Overall mean T_{el} and range	16.4 (10.4–26.6)						17.3 (10.0–32.6)					

Variation % is the difference between the highest and lowest value for a subject expressed as a percentage of the subjects mean value. Variation ratio denotes the highest monthly value divided by the lowest monthly value.

Table 3. Bioavailability (AUC in ng/ml/h) of EE

Formulation:	EE 50 + LNG 250 μg						EE 50 μg					
Subject:	1	2	3	4	5	6	1	2	3	4	5	6
Month 1	14.3	12.5	17.0	6.4	18.6	8.5	17.0	10.1	16.2	4.7	12.9	6.6
Month 2	12.1	11.8	15.8	6.4	17.3	8.0	15.0	12.5	12.7	—	13.4	7.2
Month 3	15.2	12.9	14.7	—	—	9.0	14.0	8.8	13.6	4.8	10.3	8.4
Mean	13.9	12.4	15.8	6.4	18.0	8.5	15.3	10.5	14.2	4.75	12.2	7.4
Variation %	22.3	8.9	14.6	0	7.2	11.8	19.6	35.2	24.6	10.5	21.3	24.3
Variation ratio	1.3	1.1	1.2	1.0	1.1	1.1	1.2	1.4	1.3	1.0	1.3	1.3
Overall mean AUC and range	12.5 (6.4–18.6)						11.1 (4.7–17.0)					

For details see the legend to Table 2.

(e.g. subjects 2, 4 and 5, EE 50 + LNG 250 μg), whereas for others (e.g. subjects 2, 5 and 6, EE 50 μg) the variability was much larger. As for T_{el} some subjects (e.g. 2, 5 and 6) show large monthly variations with one formulation but not with the other. The intrasubject variation in AUC tended to be higher when EE was administered alone and for the mean variation % the difference between EE + LNG and EE alone was statistically significant, but this may be a chance finding. The intersubject variability for both AUC and T_{el} was higher than the intrasubject variability and particularly so for AUC (Table 4).

There were no significant differences between the mean values for T_{el} or AUC whether EE was given alone or with LNG.

DISCUSSION

Many problems exist in determining pharmacokinetic parameters for the contraceptive steroids and some of these have been outlined [4] using LNG as an example. Similar problems also exist for EE and are further complicated by the fact that EE, but not LNG, undergoes both a first-pass effect and an enterohepatic circulation. This report concentrates on two pharmacokinetic parameters, the rate of elimination of the steroid and its bioavailability, which are important in that they can readily be calculated from serum concentrations of the steroid and are easily comprehensible. It seems likely that the variability observed with these two measurements also applies to other pharmacokinetic parameters.

Drug disposition and metabolism may be influenced by many factors, e.g. age, sex, body mass, smoking, exercise, environmental, nutritional and genetic factors, treatment with other drugs, presence of pathology etc. It is not too surprising therefore that there should be large differences between subjects. Such large differences in the metabolism of the contraceptive steroids, based upon measurement of pharmacokinetic parameters have been documented for norethisterone [12], LNG [4], for the newer gestogens desogestrel and gestodene [13], and for EE [7, 8, 11, 13]. The present results for EE are in agreement with these latter reports. Less information is available regarding intrasubject variability but recently such variability for LNG [4] and EE [11, 14] has been described and it is of interest that in both these studies, intrasubject variation was almost as large as intersubject variation. In the present study intrasubject variability was, as expected, less than intersubject variability, about 50% for T_{el} and 20% for AUC. For both T_{el} and AUC the intersubject range of values (about 3-fold) was less than that reported previously [3] but this may partly be due to the smaller number of subjects in the present study. The intrasubject range of values was <2-fold. The larger intersubject variation observed in AUC than in T_{el} is to be expected since T_{el} is only one of the factors influencing AUC. There was only a weak correlation ($r = 0.37$) between T_{el} and AUC. The intrasubject variability may become greater as the number of estimations per subject increases. In the present study, on three occasions values were available only for two study periods instead of three; for these three occasions the mean intrasubject variation (%) was 20.5% for T_{el} and 5.9% for AUC compared with values of 35.4 and 20.3% for the three study periods.

The factors responsible for the large intrasubject variability are not known. In a study of intersubject variations in serum EE concentrations in 93 women [15], 72% of the variation was unexplained on the basis of time since administration, day of cycle, age, race, height, weight, blood pressure, smoking or use of oral contraceptives. On this and other evidence the main determinant of intersubject variability is considered to be genetic. The demonstration in the present study and in others [4, 11] that intrasubject variability may be only slightly lower than intersubject variability would seem to invalidate this conclusion. However, as shown in Table 4, the quantitative relationship between intra and intersubject variability depends on the statistical method used to analyse the results. Using, for example, analysis of variance intrasubject variability was a smaller proportion of intersubject variability than observed using the other computations shown in Table 4.

Apart from being an interesting aspect of drug metabolism, intrasubject variability may have a number of important implications with respect to oral contraceptive (OC) use, of which two examples can be given. Firstly in order to reduce the incidence of side-effects in women using OCs it has been suggested that the doses administered should be tailored to the individual woman. Clearly, large intrasubject variations render such an approach unfeasible. Secondly, whilst the mechanism of breakthrough bleeding experienced in OC users has

not been elucidated, it seems that hormonal support to the endometrium is important. When this is lost at the end of a cycle of treatment, bleeding occurs within 2–3 days. Such support might be lost and breakthrough bleeding occur, if due to intrasubject variability, the women were to experience 2–3 days of low bioavailability and an increased rate of elimination of the OC with a consequent marked decrease in hormonal effect.

The mean value of T_{el} in the present study, based upon all 33 estimates, was 16.8 h. This is slightly longer than the mean values reported by others (see Table 1), partly due to the method of calculation, but probably mainly due to the present values being obtained under steady-state conditions compared to the single dose administrations in most other studies. Comparison of AUC values is more difficult since these values depend on the method used for measuring serum EE concentrations. Most studies have measured conjugated EE and the mean values reported for AUC for a 50 μg dose (Table 1) vary from 0.75 to 1.06 ng/ml/h. Serum concentrations of total EE as measured in the present study are about 10-fold those of the unconjugated steroid and, consequently, AUC for total EE is correspondingly higher than for the unconjugated [16]. Thus, the range of values (4.7–18.6 ng/ml/h) and the mean (11.6 ng/ml/h) found in the present study are in agreement with reported values for unconjugated EE.

In addition to providing information on subject variability, the present study also provides information on T_{el} and AUC for EE under steady-state conditions. It has been suggested [13] that co-administration of a gestogen may influence the metabolism of EE; serum levels of EE were higher in women taking an OC containing EE and gestodene than in those using the same dose of EE with desogestrel. In the present study LNG did not affect EE metabolism; mean values for T_{el} and AUC were 17.3 ± 5.5 h and 11.1 ± 3.8 ng/ml/h, respectively, when EE was administered alone compared with 16.4 ± 4.8 h and 12.5 ± 3.9 ng/ml/h when given with LNG. These values are not statistically significantly different. Conversely, EE will affect the metabolism of LNG. T_{el} for LNG was 19.3 ± 4.2 h when administered alone, but significantly higher (30.0 ± 11.2 h) when given with EE. The possible explanations for this difference have been considered [4]. In a previous study [12], it was shown that there was no correlation between the rate of metabolism of EE and norethisterone when the steroids were administered together in an OC. Similarly, there was only a weak correlation ($r = 0.21$; not statistically significant) between T_{el} for LNG and EE in the present study.

The considerable (up to 350%) intrasubject month-to-month variability probably reflects day-to-day variations. In this study sampling was performed with the subjects under fairly basal conditions, so it might be expected that variability would be much larger in women using contraceptive steroids on a long-term basis since changes in the subject's physiology and lifestyle over the period of use may accentuate the variability.

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