Plasma concentrations of ethynyl oestradiol and norethindrone after oral administration to women

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Summary. Plasma concentrations of ethynyl oestradiol and norethindrone in women were measured by radioimmunoassays after oral administration of 50 µg and 1000 µg respectively. The maximum values were obtained 1 h after administration. The calculated half-life was 6½ h for ethynyl oestradiol and 7 h for norethindrone. At most 2–3 % of the administered dose was present in the plasma at 1 h and had decreased to about 0.5 % by 24 h.

Introduction

The synthetic steroids norethindrone (17 α -ethynyl-17 β -hydroxy-4-oestren-3-one) and ethynyl oestradiol (17 α -ethynyl-1,3,5(10)-oestratriene-3,17 β -diol) are the most widely used components of hormonal contraceptives. Studies on the metabolism of these compounds in women have demonstrated that the ethynyl group in norethindrone remains intact (Kamyab, Fotherby & Littleton, 1967; Kamyab, Fotherby & Kloppe, 1968; Fotherby, 1974), but that the ring A is transformed into several reduced products, i.e. 17 α -ethynyl-17 β -hydroxy-5 α -oestrane-3-one and 17 α -ethynyl-5 α -oestrane-3 β ,17 β -diol (Murata, 1968), 17 α -ethynyl-5 α -oestrane-3 α ,17 β -diol and 17 α -ethynyl-5 β -oestrane-3 α ,17 β -diol (Gerhards, Hecker, Hitze, Nieuweboer & Bellmann, 1971; Stillwell, Horning, Horning, Stillwell & Zlatkis, 1972). Following oral administration of [4-¹⁴C]ethynyl oestradiol to women, 30–34 % of the dose is excreted in the urine and 22 % in the faeces; most of the radioactivity of the material, obtained as free fractions or after hydrolysis, was found to be unchanged (Reed, Fotherby, Steele & Addison, 1972).

The present study was an investigation of the plasma concentrations of unmetabolized norethindrone and ethynyl oestradiol at different times after oral administration of these steroids to women. A preliminary account has appeared elsewhere (Kincl, Pasqualini & Hill, 1975).

Materials and Methods

Subjects. The plasma was obtained from 3 groups of volunteers who received three different preparations. Group A: 6 women each received 1 tablet of Norinyl-1 (Syntex) containing 1 mg norethindrone + 0.05 mg mestranol (3-methyl ether of ethynyl oestradiol). Group B: 6 women each received 1 tablet of Norlestrin-1 (Parke Davis) containing 1 mg norethindrone acetate and 0.05 mg ethynyl oestradiol. Group C: 6 women each received 1 tablet of Mead Johnson 1-50 containing 1 mg norethindrone and 0.05 mg ethynyl oestradiol. The tablet was ingested at 0 h and blood samples were collected 0, ½, 1, 3, 5, 8 and 24 h later. Plasma was separated and frozen until assayed.

Extraction of plasma and assay. Each 5 ml plasma to be extracted was processed as follows: 10,000 d/min of [³H]norethindrone (sp. act. 20 Ci/mmol: New England Nuclear, Boston, U.S.A.) or 10,000 d/min [³H]ethynyl oestradiol (sp. act. 46 Ci/mmol: a generous gift from Dr C. Cook, Research Triangle Institute, North Carolina, U.S.A.) were added to extraction tubes for calculation of losses; the solvent was evaporated under nitrogen. Plasma (5 ml) was added to 50 ml methylene chloride and

extracted in a rotary extractor at room temperature for 60 min; the extraction procedure was repeated 3 times. The extracts were combined, dried with anhydrous sodium sulphate and the solvent was removed in a rotary evaporator *in vacuo*. The original extract was dissolved in distilled, deionized water (4 ml) and extracted four times with 8 ml (each time) methylene chloride. The solvent was dried with anhydrous sodium sulphate, evaporated *in vacuo* and dissolved in 0.5 ml absolute ethanol (i.e. 100 μ l of the final solution corresponded to 1 ml plasma). A 50- μ l aliquot was counted directly to determine the recovery. Each plasma sample was assayed by taking several aliquots (e.g. 2, 5 or 10 μ l to assay norethindrone and 20, 40 or 50 μ l for ethynyl oestradiol). All samples were in duplicate or triplicate. The solvent (absolute ethanol) was removed by evaporation under nitrogen.

Antisera to norethindrone (raised in rabbits) and ethynyl oestradiol (raised in sheep) were generously provided by Dr C. Cook (Research Triangle Institute, North Carolina) and were used at a dilution of 1/4000 for the norethindrone assay and 1/8000 for the ethynyl oestradiol assay, each giving about 65% binding. The radioimmunoassays were carried out as described by Pasqualini *et al.* (1976). The recovery was $76 \pm 16\%$ (S.D., $n = 112$) and the coefficient of variation for 42 samples in the range of 25–1000 pg was 7 ± 1.1 (S.E.)% for norethindrone and $8 \pm 1.4\%$ for ethynyl oestradiol. Blank values obtained with the plasma collected at 0 h gave values of 10–20 pg norethindrone/ml and 5–15 pg ethynyl oestradiol/ml and were deducted from the experimental values.

Data for each hormone were evaluated with a time $\times$ experiment $\times$ subject design analysis of variance. Significant factors were further analysed with Duncan's New Multiple Range Test. All data met the assumption of variance homogeneity when tested by maximum variance divided by total variance (Bliss, 1967; Bruning & Kintz, 1968). Biological half-life was estimated by planimetric determination of the area under the curve for each hormone.

Results

The progestagenic component

Two of the preparations of norethindrone tested (Norinyl-1 and Mead Johnson 1-50) contained the free alcohol whereas the third (Norlestrin-1) contained the corresponding 17-acetate at the same dose level (1 mg). The 17-acetate cleaves readily following oral administration with the formation of a free alcohol (Fotherby, 1974). The present results support these findings: in this study we found no differences in plasma levels between Norinyl-1 and Norlestrin-1 (Table 1). The mean values changed significantly over the 24-h period ($F = 9.27$, d.f. = 5.25, $P < 0.005$). The highest absolute mean value occurred 1 h after administration but Duncan's test indicated that there was no significant change in plasma level over the first 5 h. The difference between 5- and 24-h values was highly significant, but significant plasma levels persisted for at least 24 h. The biological half-life was about 6½ h.

The oestrogenic component

Two of the preparations tested (Mead Johnson 1-50 and Norlestrin-1) contained 0.05 mg ethynyl oestradiol and the third (Norinyl-1) contained 0.05 mg of the corresponding 3-methyl ether, mestranol. The ethynyl oestradiol antibody reacted with mestranol at a displacement of only about one-third that obtained with ethynyl oestradiol, and it was therefore necessary to use larger aliquots to obtain values on the useful portion of the curve. Statistical analysis of the data revealed no significant difference between values obtained with the two substances but because it is not known to what extent mestranol is converted into ethynyl oestradiol the former values were excluded from the composite means (Table 2). The mean plasma levels changed significantly over the 24-h period ($F = 8.06$, d.f. = 5.25, $P < 0.005$). As was the case with norethindrone, the highest absolute value occurred at 1 h. The data show that a high level of ethynyl oestradiol is maintained for at least 3 h and a decrease occurs between 3 and 5 h to a level which is maintained for at least 24 h. The biological half-life for ethynyl oestradiol was estimated to be about 7 h.

Table 1. Plasma levels (pg/ml) of norethindrone in women after oral administration

Exp. No.	Patient No.	Time (h)						
		0	$\frac{1}{2}$	1	3	5	8	24
A	1	N.D.	9610	10065	4890	3630	2430	1940
	2	N.D.	11960	16250	4860	2240	500	N.D.
	3	N.D.	3280	5600	5100	4940	2500	2460
	4	N.D.	1800	2830	2855	1590	1570	380
	5	N.D.	770	1850	7630	2710	830	N.D.
	6	N.D.	830	1120	5070	6090	1175	540
B	1	N.D.	9610	12905	5470	4205	2430	730
	2	N.D.	8490	9535	8270	5220	3310	950
	3	N.D.	490	2040	4390	5260	1745	N.D.
	4	N.D.	9080	9080	8960	9120	4350	600
	5	N.D.	11060	10040	4420	3230	2300	460
	6	N.D.	4950	8830	12879	10260	4360	1170
C	1	N.D.	4840	6800	4785	2800	3020	740
	2	N.D.	5170	10608	5250	4840	110	N.D.
	3	N.D.	940	1660	4090	1850	1160	560
	4	N.D.	6430	5280	4610	4610	4330	2480
	5	N.D.	9880	12450	13260	10710	9230	4230
	6	N.D.	1330	3630	3230	4230	2900	950
Mean $\pm$ S.E.M.			7260 $\pm$ 1058					1015 $\pm$ 240

N.D. = not detectable.

Table 2. Plasma levels (pg/ml) of ethynyl oestradiol in women after oral administration

Exp. No.	Patient No.	Time (h)						
		0	$\frac{1}{2}$	1	3	5	8	24
A	1	N.D.	230	300	111	95	85	N.D.
	2	N.D.	790	720	650	90	N.D.	N.D.
	3	N.D.	1320	910	240	240	N.D.	N.D.
	4	N.D.	700	1230	645	200	190	160
	5	N.D.	650	690	880	560	430	300
	6	N.D.	N.D.	N.D.	230	330	190	190
B	1	N.D.	110	150	325	240	110	90
	2	N.D.	230	730	370	110	60	N.D.
	3	N.D.	200	230	260	200	160	125
	4	N.D.	500	510	360	280	210	80
	5	N.D.	480	940	320	300	300	95
	6	N.D.	50	50	270	690	190	160
C	1	N.D.	270	450	450	310	260	190
	2	N.D.	65	120	70	N.D.	N.D.	N.D.
	3	N.D.	60	100	110	120	120	55
	4	N.D.	1060	890	340	120	100	90
	5	N.D.	300	300	420	310	100	N.D.
	6	N.D.	580	685	380	340	330	220
Mean $\pm$ S.E.M.*			422 $\pm$ 128					86 $\pm$ 28

N.D. = Not detectable. * Excluding Group A values (see text).

Discussion

The original ratio between norethindrone and ethynyl oestradiol in the tablets was 20:1. The mean ratio based on observed values in plasma for all time periods was 17.7 ± 0.9 (S.E.M.), perhaps indicating that both components may have similar excretion rates. The regression equations of the two compounds were similar for the interval between 1 and 8 h. To adjust for the difference in scale, the mean plasma level at 1, 3, 5 and 8 h for each compound was divided by the value at 1 h, and the data were expressed as proportions of the peak plasma level. The regression equations and proportion of the variance explained by linear regression were $y = 1.103 - 0.89x$ and 0.977 for norethindrone and $y = 1.069 - 0.099x$ and 0.969 for ethynyl oestradiol. The fact that the slopes of the two lines did not differ significantly ($t = 0.86$, d.f. = 4, $P = 0.10$) further suggests that the excretion rates of the two compounds are probably similar. The lower ratio may have resulted from partial metabolism of norethindrone to ethynyl oestradiol.

One hour after administration of [4- 14 C]ethynyl oestradiol to women, Reed, Fotherby & Steel (1972) found $8.5 \pm 0.8\%$ of the radioactivity in whole blood: of this 2.45% was present as free ethynyl oestradiol, 2.25% as ester sulphates and 1.35% was obtained following enzymatic hydrolysis. If the average plasma volume in the patients studied is considered to be 2500 ml, then our results show that 2–3% of the administered dose of ethynyl oestradiol and norethindrone is present 1 h after administration, and are in agreement with the values obtained by Reed *et al.* (1972). The value at 24 h decreased to about 0.5%.

The estimation of 7 h for the biological half-life of ethynyl oestradiol is in good agreement with the data reported by Warren & Fotherby (1973), who based their calculation on the material obtained by extracting plasma with ether and assaying ethynyl oestradiol by a competitive protein-binding method using a rabbit uterine cytosol preparation (Korenman, Perrin & McCallum, 1969). These authors also found a maximum value for ether-extractable material 1 h after ingestion, a finding similar to that in the present paper. However, Warren & Fotherby calculated that the half-life 8 h after administration was 48 h; this difference could be explained by the existence of at least two components, one for the unmetabolized ethynyl oestradiol of 7–8 h and the other, much longer, for the metabolites. The half-life of 6½ h which we found for norethindrone is also in agreement with the values of 3 and 5 h found by Warren & Fotherby (1974) after oral administration of 1 mg norethindrone and using a radioimmunoassay with 125 I-labelled norethindrone.

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