RATE OF METABOLISM OF NORETHISTERONE IN WOMEN FROM DIFFERENT POPULATIONS

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ABSTRACT

The rate of metabolism of orally administered norethisterone was compared in fourteen centres by measuring plasma levels of the steroid by radioimmunoassay at varying times after oral administration of a 1 mg dose. The inter-centre differences were of the same order as the intra-centre differences. Variations in metabolism appeared not to be due to variations in body size.

Accepted for publication December 14, 1978

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INTRODUCTION

The synthetic gestagen norethisterone, or its derivatives such as the acetate and the oenanthate, are widely used as contraceptive steroids and are being utilized in many clinical trials being undertaken by the World Health Organisation. Norethisterone was one of the first synthetic gestagens to be evaluated so that a very large amount of clinical experience has been accumulated and it has proved satisfactory in toxicity tests. The network of WHO Collaborating Centres for Clinical Research in Human Reproduction and Research and Training Centres allows multicentred comparative trials of fertility regulating agents to be carried out in a number of different ethnic groups. Little is known concerning the metabolism of synthetic steroids, which may be influenced by environmental, dietary and genetic factors, in these different populations. To date, most of the investigations of the metabolism of the synthetic steroids have involved administration of the labelled compound but because of the regulations controlling the use of labelled compounds in humans, the number of studies which have been carried out have been limited. The availability of sensitive radioimmunoassays for many of the synthetic compounds in widespread use enables the concentration of the steroid in blood to be measured simply and accurately. From the analysis of timed samples, it is possible to measure one important aspect of the metabolism of the steroid, its plasma half-life. In the present investigation the rate of metabolism of norethisterone was compared in groups of women in fourteen different centres.

DESIGN OF INVESTIGATION

The study was carried out in volunteer female subjects or in women attending a family planning clinic who intended to use combined oral contraceptives; the tablet to be taken was the first of a month's supply in the latter case. Criteria for the selection of the subjects were the same as those usually employed in trials of contraceptive steroids: subjects were between 20 and 40 years of age with no evidence of abnormal thyroid, adrenal or hepatic function; they should not have used steroidal contraceptives, been pregnant or lactating, or have received steroid therapy within the preceding three months. The age, weight and height of each subject were recorded (see Table II) and also the date and duration of the last menstrual period. All subjects were studied on days 5 or 6 of the menstrual cycle and were carefully informed of the aims of the study and informed consent was obtained.

The Centres from which subjects were recruited are shown in Table I. Each Centre was required to recruit six subjects, Chandigarh recruited 7 and results were not available for 2 of the 6 subjects studied in Lusaka due to loss of samples in transit. Between 0800 h and 0900 h on the day of the study, each subject took a tablet containing 1 mg norethisterone and 50 µg ethynyloestradiol orally. Prior to taking the tablet and at 0.5, 1, 2, 4, 8, 12 and 24 h after administration of the tablet, a venous blood sample (about 10 ml) was taken. The blood was allowed to clot and then centrifuged

to obtain serum. The serum samples were stored at -20°C until assayed. The concentration of norethisterone in each sample was estimated by radioimmunoassay(1).

For testing the significance between the slopes of the regression lines, the procedure described by Armitage (2) was used. The ponderal index (height in cm divided by the cube root of the weight in Kg) was calculated as described by Khosla and Lowe (3).

RESULTS AND DISCUSSION

The mean values for the serum norethisterone concentration obtained on the samples from each centre are shown in Table I. The peak concentration was reached in plasma between 1 and 2 h after administration in all centres except Singapore and Seoul where the peak occurred between 2 and 4 hours. After the peak there was a marked decrease in the serum norethisterone concentrations, but even 24 h after administration, significant amounts of the steroid were still present in serum, mean values ranging from 275 pg/ml in Stockholm to 935 pg/ml in Mexico. Values in excess of 1000 pg/ml at 24 h were recorded for 2 of the subjects in Bahia, 2 in Bombay, 1 in Los Angeles, 2 in Mexico and 3 in Singapore whereas in Stockholm all of the values recorded at 24 h were less than 325 pg/ml. As shown by the standard deviations for the mean values, there were considerable variations between the values obtained at any particular time from the subjects recruited in any one centre. The values obtained in the present study are similar to those reported for British subjects (1). As expected there was a highly significant decrease ($P < 0.001$) of the serum norethisterone levels with time from 2 to 24 h (Table I), the mean correlation coefficient (R) varying from 0.80 (Los Angeles) to 0.96 (Stockholm) for all centres except Bahia which showed a much lower value (0.67)

Since the decrease in serum norethisterone concentrations from 2 to 24 h after administration appeared to be exponential, approximately straight lines were obtained when the logarithms of these values were plotted against time. The slope of the line represents the rate of metabolism of the steroid, the steeper the slope the more rapid the metabolism. Thus it was possible to compare the rate of metabolism of the steroid in the different centres by calculating the regression equation for the straight line best fitting the values for the serum concentration of norethisterone from 2 to 24 hours after administration when these are plotted semilogarithmically. The regression equations for these lines are shown in Table II. The slope of the regression line (indicated by the constant A) varied from the highest value of 0.055 in Stockholm to the lowest value of 0.032 in Bahia. The mean value for Stockholm was significantly higher than that of all other centres and that for Canada (0.048) was significantly higher than that of Bangkok and other centres with a value of less than 0.043. Differences between the centres were statistically significant when the difference between the values for A was 0.005 or more, with the exception of the difference between Singapore and Mexico.

Table I. Serum norethisterone concentrations after administration of 1 mg norethisterone orally. (Values are mean ± SD in pg/ml; number of subjects per centre in parentheses. R is the correlation for serum norethisterone concentration and time after administration.)

Centre	Time (h) after administration of dose							R
	0.5	1	2	4	8	12	24	
Alexandria(6)	2064 ± 1606	3763 ± 1567	4094 ± 1161	3134 ± 976	1144 ± 467	1000 ± 322	408 ± 191	0.89
Bahia (6)	--	2664 ± 1850	3789 ± 1262	3013 ± 431	2373 ± 1025	1637 ± 980	848 ± 489	0.67
Bangkok (6)	2279 ± 411	3013 ± 1324	2602 ± 518	2776 ± 808	1695 ± 436	804 ± 194	340 ± 168	0.91
Bombay (6)	1972 ± 1791	3465 ± 1686	4381 ± 764	3178 ± 797	1930 ± 385	1197 ± 291	740 ± 295	0.92
Chandigarh (7)	2337 ± 2097	3457 ± 1680	4344 ± 669	3604 ± 935	2139 ± 692	1403 ± 468	621 ± 146	0.92
London, Canada (6)	2730 ± 1124	4486 ± 941	4282 ± 841	3452 ± 772	1983 ± 671	1315 ± 447	402 ± 141	0.95
Los Angeles (6)	2398 ± 1268	4059 ± 1083	3586 ± 1461	2747 ± 660	1004 ± 1314	1270 ± 970	598 ± 513	0.80
Lusaka (4)	1674 ± 588	3866 ± 782	3749 ± 653	3279 ± 1194	2053 ± 708	1461 ± 689	534 ± 219	0.91
Mexico (6)	3906 ± 2007	5613 ± 2037	5975 ± 1749	4730 ± 2055	2569 ± 1045	1672 ± 632	935 ± 516	0.85
Santiago (6)	1629 ± 700	3156 ± 1204	4012 ± 1027	2848 ± 601	1969 ± 423	1231 ± 391	444 ± 296	0.93
Seoul (6)	1338 ± 769	2977 ± 1693	4050 ± 936	3380 ± 796	2498 ± 539	1630 ± 536	585 ± 180	0.93
Singapore (6)	2437 ± 2175	3473 ± 1375	5378 ± 1583	4806 ± 1957	2738 ± 1134	1799 ± 927	763 ± 514	0.82
Stockholm (6)	2836 ± 1349	5320 ± 787	4849 ± 885	3108 ± 835	1772 ± 521	1035 ± 291	275 ± 61	0.96
Sydney (6)	2664 ± 1869	4446 ± 2422	3546 ± 860	3343 ± 1425	2448 ± 1076	1867 ± 820	616 ± 212	0.83

Table II. Details of subjects studied and regression equations and plasma half-lives for serum norethisterone levels from 2 to 24 hours. (Values for age, height and ponderal index are mean ± SD. X = time (h), Y = log serum norethisterone concentration. Figure in parentheses denotes number of samples analysed.)

Centre	Age (y)	Height (cm)	Weight (kg)	Ponderal index	Regression line equation (Y=B-AX)	Anti-log B	Half-life (h) for period 2-8h	8-24h
Alexandria(30)	32.8 ± 8.9	161.0 ± 3.7	72.0 ± 22	39.4 ± 4.5	3.59-0.046X	3890	4.3	12.4
Bahia(30)	25.1 ± 5.0	153.5 ± 10.6	49.1 ± 7.3	41.9 ± 1.5	3.53-0.032X	3383	-	12.4
Bangkok(30)	29.0 ± 7.4	158.0 ± 4.0	52.2 ± 10	42.5 ± 2.6	3.49-0.043X	3090	8.6	10.0
Bombay (30)	24.2 ± 2.4	151.7 ± 6.1	47.5 ± 2.2	41.9 ± 1.4	3.63-0.034X	4266	5.3	12.1
Chandigarh(34)	26.9 ± 4.2	157.6 ± 3.6	51.6 ± 4.5	42.4 ± 0.9	3.67-0.039x	4677	5.9	11.3
London, Canada(30)	26.7 ± 4.4	162.7 ± 6.7	50.8 ± 1.5	43.9 ± 1.5	3.70-0.048X	5012	5.6	10.0
Los Angeles(29)	27.8 ± 4.6	159.2 ± 4.1	58.5 ± 8.2	41.1 ± 1.5	3.57-0.039X	3715	6.8	11.4
Lusaka (20)	25.7 ± 3.0	156.0 ± 1.4	59.7 ± 5.0	39.9 ± 1.4	3.64-0.040X	4365	6.6	10.8
Mexico(30)	35.0 ± 2.5	168.8 ± 3.6	57.4 ± 4.5	41.2 ± 1.1	3.74-0.037X	5495	5.2	12.6
Santiago (29)	27.7 ± 4.1	155.8 ± 2.7	53.7 ± 1.9	41.3 ± 0.8	3.65-0.045X	4467	5.9	10.3
Seoul (30)	23.3 ± 1.6	153.5 ± 3.0	46.8 ± 5.9	42.7 ± 2.2	3.68-0.039X	4786	7.8	10.2
Singapore(30)	24.3 ± 3.8	153.2 ± 3.3	47.8 ± 4.7	42.3 ± 1.3	3.78-0.043X	6026	6.1	11.1
Stockholm (30)	27.0 ± 5.7	167.8 ± 8.6	57.0 ± 8.2	43.7 ± 1.2	3.70-0.055x	5012	4.3	9.7
Sydney (29)	30.8 ± 4.6	167.3 ± 5.5	61.1 ± 11	42.7 ± 2.3	3.63-0.035X	4266	10.8	10.7

However, whereas in Stockholm, the values for the slope of the regression lines for the six subjects analysed individually were within a very narrow range (from 0.053 to 0.057), for all other centres variations in the slope between the subjects studied in that centre varied considerably. For example, the slopes varied in Alexandria from 0.034 to 0.066; in Canada, from 0.041 to 0.058; in Bombay from 0.028 to 0.043; in Chandigarh, from 0.033 to 0.052; and in Santiago from 0.032 to 0.058. Thus, with the exception of Stockholm, differences in the slope between subjects in any particular centre were as large as the inter-centre differences.

Variations in the slope of the regression line appeared not to be due to variations in body mass between the subjects (Table II). Neither in Alexandria where there was a large range in body weight of the subjects (from 42 to 100 Kg) nor in Stockholm, Canada, Chandigarh and Santiago was there any correlation between the value for the slope and the body weight or ponderal index. However, in Bombay, there was a significant correlation ($R = 0.82$, $P = 0.045$) between the values for A and ponderal index.

Another way to compare the rate of metabolism of norethisterone would be to calculate the half-life of the steroid in blood. Warren and Fotherby (1) calculated approximate half-lives for the time periods 2 to 8 h and 8 to 24 h after administration as 3 h and 5 h, respectively. Values for the half-lives, calculated in the same way, obtained in the present investigation are shown in Table II. For all except three centres (Bangkok, 8.6 h; Seoul, 7.8 h; Sydney 10.8 h) for the period 2 to 8 h, the values were within the range of 4.2 to 6.8 h. Values for the period 8 - 24 h after administration were much more consistent and all the values were within the range 9.7 to 12.9 h. The higher values for the half-lives found in the present study compared to that of Warren and Fotherby could be due to the fact that whereas all the subjects in the present study were females, male subjects were studied by Warren and Fotherby. However, it seems more likely that the difference is due to the administration of the norethisterone with 50µg ethynyloestradiol in the present study whereas Warren and Fotherby administered norethisterone alone. It has recently been shown (4) that there is a large difference in the half-life of norgestrel depending on whether it is administered with or without oestrogen.

Table II also shows the values for the constant B. The antilog of constant B represents the value for the serum norethisterone concentration at time zero, i.e. assuming that the dose was instantaneously absorbed and distributed throughout the body compartments. Mean values of B ranged from 3.49 (antilog 3090 pg/ml, Bangkok) to 3.78 (antilog 6026 pg/ml, Singapore). As with the values for the slope of the regression lines, intra-centre differences were similar to inter-centre differences. Thus, in Alexandria values for B ranged from 3.37 to 3.80 (2344-6310 pg/ml), in Stockholm from 3.57 to 3.82 (3715-6607 pg/ml), in Bombay from 3.55 to 3.67 (3311-4677 pg/ml), in Chandigarh from 3.51 to 3.78 (3267-6026 pg/ml) and in Santiago from 3.55 to 3.74 (3548-5495 pg/ml). There was no correlation between the

mean values for A and B (R = 0.14) showing that the rate of metabolism of norethisterone was not related to the initial serum concentration attained. For the various centres tested, there was no correlation between the value of B or antilog B and the ponderal index.

ACKNOWLEDGMENT

This investigation received financial support from the World Health Organisation.

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