Pergamon

0960-0760(95)00158-1

J. Steroid Biochem. Molec. Biol. Vol. 55, No. 1, pp. 129–133, 1995

0960-0760/95 $9.50 + 0.00

Urinary Excretion of 6β-Hydroxycortisol in Women During Treatment With Different Oral Contraceptive Formulations

W. Kuhnz* and B. Löfberg

Research Laboratories, Institut für Pharmakokinetik, Schering AG, 13342 Berlin, Germany

The measurement of the urinary excretion ratio of 6β-hydroxycortisol (6β-OHC)/cortisol was used as a non-invasive method to investigate possible changes in the activity of drug-metabolizing enzymes in women receiving different oral contraceptive formulations for 1 up to 3 treatment cycles. The contraceptive preparations were either levonorgestrel, gestodene or cyproterone acetate, each in combination with ethinyl estradiol, or only the progestogens levonorgestrel or gestodene. There was either no or only a small decrease in the 6β-OHC/cortisol ratio. Thus, only a minor inhibitory effect, if any, can be ascribed to the investigated contraceptive steroids *in vivo*. Previously observed differences between selected contraceptive steroids *in vitro* were not observed in the same way *in vivo*. This may be due either to the absence of a marked inhibitory activity *in vivo* or to the insufficient sensitivity of the marker 6β-OHC/cortisol to detect these changes. Another possible reason may be the considerably higher drug concentrations used in the *in vitro* studies as compared to those present in the serum of women under oral contraceptive therapy.

J. Steroid Biochem. Molec. Biol., Vol. 55, No. 1, pp. 129–133, 1995

INTRODUCTION

The measurement of urinary 6β-hydroxycortisol (6β-OHC) provides a simple and non-invasive method to determine changes in the rate of drug metabolism in man [1]. 6β-OHC is a minor metabolite of cortisol which is formed primarily by the hepatic monooxygenases and is excreted in non-conjugated form in urine. Since many drugs are metabolized by the same enzyme systems, changes in the urinary excretion of 6β-OHC provide a useful index of either enzyme induction or inhibition during or after drug treatment.

A number of studies have revealed the propensity of several synthetic steroids, used in oral contraceptive formulations, to inhibit the metabolism of certain marker compounds, like nifedipine, ethinylestradiol (EE_2), diazepam etc. when incubated together with human microsomal preparations *in vitro* [2–5]. It was, however, unclear whether this had any relevance for the clinical situation. Although there are numerous reports on the influence of oral contraceptive treatment on the hepatic clearance of several probe drugs in women, recent studies seemed to indicate that as far as the urinary excretion of 6β-OHC is concerned, there is no change in women using oral contraceptives [6, 7].

It was the aim of this study, to investigate whether there is any change, in particular a reduction, in the urinary excretion ratio of 6β-OHC/cortisol in women, who have been treated either with different combination oral contraceptives or with two different progestogens alone, over at least one treatment cycle.

MATERIAL AND METHODS

Origin of urine samples

The urine samples were obtained during 5 different clinical trials from healthy, young women. All women had not taken oral contraceptives for at least 2 months prior to their participation in these studies. The women received single doses of different contraceptive formulations during a pre-treatment cycle and, after a wash-out phase, treatment was continued over a period of 1 to 3 cycles. This design allowed for the intraindividual comparison of single and multiple dose pharmacokinetics of the different progestogens. Urine samples were collected from each woman prior to treatment, following single dose administration and at several occasions during the treatment period. Thus,

*Correspondence to W. Kuhnz.
Received 15 Mar. 1995; accepted 16 June 1995.

an effect of both, single dose and multiple dose treatment with the contraceptive steroids on the urinary 6β-OHC/cortisol ratios could be assessed. In those trials where tri-step preparations were administered (trials I and II), one urine sample was collected during each dose step per cycle. In all studies, urine samples were collected on days 1 and 21 of each treatment cycle, in order to detect any treatment-dependent effect within this time period. Details of the study protocols and the outcome of these studies with regard to the pharmacokinetic parameters of the active ingredients have been described in separate publications [8–12].

Trial I. 14 women received a single oral dose of 0.125 mg levonorgestrel (LNG) together with 0.03 mg EE_2 which represents the highest LNG content of the tri-step formulation administered (Triquilar®, Schering AG). After a wash-out period of 1 week, the same women received the contraceptive formulation over a treatment period of 3 cycles according to the following schedule: days 1–6, 0.05 mg LNG + 0.03 mg EE_2; days 7–11, 0.075 mg LNG + 0.04 mg EE_2; days 12–21, 0.125 mg LNG + 0.03 mg EE_2.

Each treatment cycle was separated by a drug-free interval of 1 week. Urine samples were collected for 24 h on the following days:

Pre-treatment cycle: prior to (day 20), as well as after single dose administration on day 21.
Treatment cycles 1 and 3: days 1, 7 and 21.

Trial II. 14 women received a single oral dose of 0.1 mg gestodene (GEST) together with 0.03 mg EE_2 which represents the highest GEST content of the tri-step formulation administered (Milvane®, Schering AG). After a wash-out period of 1 week, the same women received the contraceptive formulation over a treatment period of 3 cycles according to the following schedule: days 1–6, 0.05 mg GEST + 0.03 mg EE_2; days 7–11, 0.07 mg GEST + 0.04 mg EE_2; days 12–21, 0.1 mg GEST + 0.03 mg EE_2.

Each treatment cycle was separated by a drug-free interval of 1 week. Urine samples were collected for 24 h on the following days:

Pre-treatment cycle: prior to (day 20), as well as after single dose administration on day 21.
Treatment cycles 1 and 3: days 1, 7 and 21.

Trial III. 15 women received a single oral dose of 2.0 mg cyproterone acetate (CPA) together with 0.035 mg EE_2 (Diane-35®, Schering AG) and, after a wash-out period of 1 week, the same women received the contraceptive formulation over a treatment period of 3 cycles. Each treatment cycle was separated by a drug-free interval of 1 week. Urine samples were collected for 24 h on the following days:

Pre-treatment cycle: prior to (day 20), as well as after single dose administration on day 21.
Treatment cycles 1 and 3: days 1, 10 and 21.

Trial IV. 12 women received a single oral dose of 0.15 mg LNG and, after a wash-out phase of 1 week, the same dose of LNG, once daily for a whole treatment cycle. Urine samples were collected for 24 h on the following days:

Pre-treatment cycle: prior to (day 20), as well as after single dose administration on day 21.
Treatment cycle 1: days 1 and 21.

Trial V. 12 women received a single oral dose of 0.075 mg GEST and, after a wash-out phase of 1 week, the same dose of GEST, once daily for a whole treatment cycle. Urine samples were collected for 24 h on the following days:

Pre-treatment cycle: prior to (day 20), as well as after single dose administration on day 21.
Treatment cycle 1: days 1 and 21.

The volumes of all urine samples were measured and aliquots of about 100 ml were stored deep frozen at −20 C until analysis.

Analytical methods

The concentrations of 6β-OHC and cortisol in urine samples were measured in duplicate by specific RIA. For the determination of 6β-OHC, an antiserum raised in rabbits was obtained from Professor Park (University of Liverpool, U.K.). The cross reactivity of the antiserum with cortisol was 5–7%. Further details of the characteristics of the antiserum are described elsewhere [13]. ^{3}H-labeled 6β-OHC (6β,11β,17α,21-tetrahydroxy-4-pregnen-3,20-dion-[1,2(n)-$^3H_2$]) (4.76 GBq/mg) (Amersham) was used as tracer. The radiochemical purity of the tracer was >99%. The urine samples were diluted 1:100 in BSA-buffer and 0.1 ml incubated with the antiserum (dilution 1:1500 in the assay) and approx. 5000 cpm of tracer. Incubation time was 16 h at 4°C. Assay quality was assessed by the inclusion of 0.1 ml aliquots of quality control samples, containing 1500, 3000 and 5000 pg/ml, respectively. Inter-assay precision was between 5 and 15%. The deviation of measured concentrations of 6β-OHC from nominal values was between 1 and 11%.

The concentrations of free cortisol in the urine samples were measured in duplicate after extraction into methylene chloride using a commercially available RIA (COAT-A-COUNT® Cortisol, Diagnostic Products Corp., Los Angeles). Three quality control samples with nominal concentrations of 47, 105 and 251 ng/ml, respectively, were included in each assay. The mean measured concentrations in a total of 16 assays were 40.9 ± 2.5, 99.1 ± 4.3 and 245.3 ± 12.6 ng/ml, respectively. Inter-assay precision was between 4 and 6%, the deviation of measured from nominal values was between 2 and 13%.

Table 1. Urinary excretion (μg/24 h) of 6β-OHC and cortisol prior to (pre) and during treatment of young women with the tri-step LNG-containing formulation (mean ± SD)

Cycle	Day	6β-OHC (μg/24 h)	Cortisol (μg/24 h)	6β-OHC/cortisol
Pre	20	217 ± 103	34.5 ± 17.1	6.4 ± 1.0
Pre	21	206 ± 80	27.7 ± 9.4	7.8 ± 2.9
One	1	233 ± 65	29.8 ± 8.0	8.3 ± 3.2
One	7	275 ± 77	37.7 ± 15.0	8.5 ± 4.9
One	21	200 ± 87	24.2 ± 8.3	8.4 ± 2.7
Three	1	224 ± 81	35.3 ± 14.6	6.9 ± 2.4
Three	7	213 ± 67	34.4 ± 10.4	6.6 ± 2.3
Three	21	214 ± 66	33.0 ± 12.4	6.8 ± 1.8

Table 3. Urinary excretion (μg/24 h) of 6β-OHC and cortisol prior to (pre) and during treatment of young women with an oral contraceptive formulation containing 2.0 mg CPA and 0.035 mg EE_2 (mean ± SD)

Cycle	Day	6β-OHC (μg/24 h)	Cortisol (μg/24 h)	6β-OHC/cortisol
Pre	20	208 ± 53	34 ± 13	6.6 ± 2.0
Pre	21	211 ± 99	37 ± 26	6.6 ± 2.7
One	1	182 ± 58	33.5 ± 10.9	5.7 ± 1.8
One	10	198 ± 69	40.6 ± 13.2	5.1 ± 1.9
One	21	201 ± 116	35.8 ± 11.2	5.7 ± 2.0
Three	1	173 ± 70	30.8 ± 11.8	6.1 ± 2.3
Three	10	195 ± 69	34.9 ± 13.5	6.1 ± 2.7
Three	21	193 ± 159	32.9 ± 14.2	6.0 ± 2.7

Data evaluation

From the individual concentrations of 6β-OHC and cortisol on the one hand and the individual 24 h urine volumes on the other hand, the 24 h urinary output of both steroids was calculated and expressed as μg excreted/day. Instead of using absolute 6β-OHC values, the ratio of 6β-OHC to free cortisol was used as an index for changes in the monooxygenase activity, in order to correct for minor day to day variation in the cortisol production.

Statistical analysis

The 6β-OHC/cortisol ratios which were calculated for each subject prior to drug administration (day 21, pretreatment cycle) and following the last drug intake at the end of the treatment period (day 21, cycle one or day 21, cycle three), were compared by the two-sided paired Student t-test (significance level $P = 0.05$).

RESULTS

The urinary excretion of 6β-OHC and cortisol as well as the corresponding concentration ratios determined during the treatment with different oral contraceptive formulations are presented in Tables 1–5. In urine samples obtained from study I, the mean 6β-OHC/cortisol ratio prior to drug treatment was found to be 6.4 ± 1.0 and this was not different from the one measured at the end of 3 treatment cycles (6.8 ± 1.8). In samples obtained from study II, there was a small but significant decrease in the mean 6β-OHC/cortisol ratio from 7.5 ± 2.1 prior to treatment to a value of 6.1 ± 2.1 on the last day of cycle three. In samples from trial III, there was no statistically significant difference between the ratios determined prior to treatment (6.6 ± 2.0) and at the end of a 3 months treatment period (6.0 ± 2.7), respectively. When LNG was administered at a daily dose of 0.15 mg over a period of 1 cycle (trial IV), a significant decline from pretreatment values of 7.5 ± 2.6 to a value of 5.8 ± 1.0 at the end of the treatment period was observed. There was no difference, however, between the mean pretreatment ratio (4.0 ± 1.5) and the one determined after 1 month of daily oral intake of 0.075 mg GEST (4.5 ± 2.9) during trial V.

Table 2. Urinary excretion (μg/24 h) of 6β-OHC and cortisol prior to (pre) and during treatment of young women with the tri-step GEST-containing formulation (mean ± SD)

Cycle	Day	6β-OHC (μg/24 h)	Cortisol (μg/24 h)	6β-OHC/cortisol
Pre	20	284 ± 93	39.2 ± 13.1	7.5 ± 2.1
Pre	21	232 ± 75	36.6 ± 18.3	6.8 ± 1.9
One	1	269 ± 69	34.6 ± 12.1	8.2 ± 2.1
One	7	264 ± 67	41.1 ± 15.4	7.0 ± 2.1
One	21	234 ± 65	38.4 ± 15.5	6.6 ± 1.9
Three	1	283 ± 142	53.0 ± 49.8	6.6 ± 2.3
Three	7	281 ± 90	42.2 ± 21.6	7.4 ± 2.3
Three	21	212 ± 53	39.0 ± 18.2	6.1 ± 2.1

DISCUSSION

It has been shown that isozymes of the *P*450IIIA family are involved in the 6β-hydroxylation of endogenous steroids like cortisol, as well as in the metabolism of steroid hormones which are present in oral contraceptive formulations [14, 15]. Thus, the 6β-OHC/cortisol ratio should be a suitable marker to indicate changes in the activity of *P*450IIIA [16]. Principally, this applies to both, enzyme induction as well as enzyme inhibition, although the majority of applications which have been described so far include enzyme inducing agents. There have been indications that the 6β-OHC/cortisol ratio may be less sensitive in detecting an enzyme inhibition [17].

Table 4. Urinary excretion (μg/24 h) of 6β-OHC and cortisol prior to (pre) and during treatment of young women with 0.15 mg of LNG (mean ± SD)

Cycle	Day	6β-OHC (μg/24 h)	Cortisol (μg/24 h)	6β-OHC/cortisol
Pre	20	238 ± 79	35 ± 18	7.5 ± 2.6
Pre	21	217 ± 88	37 ± 17	6.2 ± 2.3
One	1	198 ± 66.5	31 ± 10	6.6 ± 1.9
One	21	184 ± 75.4	32 ± 11	5.8 ± 1.0

Table 5. Urinary excretion (μg/24 h) of 6β-OHC and cortisol prior to (pre) and during treatment of young women with 0.075 mg of GEST (mean ± SD)

Cycle	Day	6β-OHC (μg/24 h)	Cortisol (μg/24 h)	6β-OHC/cortisol
Pre	20	201 ± 74	54.6 ± 26.4	4.0 ± 1.5
Pre	21	169 ± 56	50.4 ± 28.5	3.8 ± 1.5
One	1	178 ± 97	56.0 ± 34.0	3.7 ± 2.3
One	21	176 ± 89	42.0 ± 11.0	4.5 ± 2.9

Several *in vitro* studies using human microsomal preparations have shown that a number of synthetic steroid hormones can inhibit cytochrome *P*450-dependent enzymes, including *P*450IIIA [2–5]. The highest inhibitory activity *in vitro* was ascribed to GEST, whereas LNG and other progestogens as well as the estrogen EE_2 turned out to be of less inhibitory potency. Since the results of *in vitro* studies cannot directly be extrapolated to the clinical situation, it was necessary to investigate whether the *in vitro* results had a clinical correlate or not. One way of approaching this problem is to examine the clearance of probe drugs in women prior to and during oral contraceptive therapy. In one study, the influence on the clearance of antipyrine was investigated for two combination oral contraceptives differing only in the progestogenic component [18]. It was found that although the two progestogens involved (GEST and desogestrel) had shown different inhibitory activity *in vitro*, there was no difference in their impact on the clearance of the probe drug *in vivo*.

Another approach to assess possible differences between contraceptive steroids in their ability to inhibit *P*450-dependent enzymes in women, is to compare the urinary excretion ratios of 6β-OHC/cortisol. In a recently published study, the authors found no difference in the 6β-OHC excretion nor the 6β-OHC/cortisol ratio between women using a GEST-containing preparation and those using a desogestrel-containing preparation for a period of 6 months [7]. In another study, comparing single oral doses of EE_2, EE_2 plus GEST and EE_2 plus desogestrel, there was also no difference in the urinary excretion of 6β-OHC [6].

In the present study, we have not only included different combination oral contraceptives, but we also investigated the effect of treatment with progestogens alone on the urinary 6β-OHC/cortisol excretion ratio. In addition, the possible influence of single dose and multiple dose administration of the contraceptive formulations could be assessed. We observed a small, but significant decrease in women who received LNG but not in those who received GEST. When the corresponding combination oral contraceptive formulations were administered, however, there was no significant change in the 6β-OHC/cortisol excretion ratios during treatment with the LNG-containing formulation, but there was a small difference during treatment with the GEST-containing formulation. It should be noted that the total dose of EE_2 administered during the treatment period was the same for both combination preparations. Treatment with an oral contraceptive containing cyproterone acetate and EE_2 had no influence on the 6β-OHC/cortisol excretion in urine.

It has been shown previously that serum levels of CBG did not change when either LNG or GEST were administered alone [11, 12]. During treatment with the EE_2-containing combination oral contraceptives an about 2-fold increase in the CBG levels occurred between the first and the last day of a treatment cycle [8–10]. Since cortisol binds with high affinity to CBG, one could suspect an influence on the CBG levels on the rate of metabolism of cortisol affecting the 6β-OHC/cortisol ratio. However, this seems not to be the case, since although CBG remained unchanged during repeated treatment with either LNG or GEST, in the former case a change in the 6β-OHC/cortisol ratio was observed. During treatment with the combination oral contraceptives containing LNG or CPA, on the other hand, there was no decrease in the 6β-OHC/cortisol ratio despite a marked increase of CBG.

The outcome of this study can be summarized and interpreted as follows. Firstly, during treatment with 5 different oral contraceptive preparations, there was either no or only a small change in the urinary 6β-OHC/cortisol excretion ratio. Thus, even if there was an inhibitory effect on the cytochrome *P*450 enzymes *in vivo*, it can only be a minor one. Secondly, it is difficult to understand why on the one hand LNG alone should have an effect on the 6β-OHC/cortisol ratio but not when administered together with EE_2 and why on the other hand, just the opposite was true for GEST. Looking at the variability of the 6β-OHC/cortisol excretion ratio during each treatment period on the one hand and on the small changes observed in the mean values of this parameter prior to and after treatment on the other hand, significance might have been obtained simply by chance. Thirdly, the present results do not agree with the *in vitro* findings, which ascribed a markedly higher inhibitory potency to GEST relative to LNG [2–5]. This again could either be due to a significance reached by chance, or it would question the relevance of an extrapolation of *in vitro* results to the clinical situation.

In conclusion, the present study has shown that prolonged treatment of young women with different oral contraceptive preparations had either no or only a marginal influence on the urinary excretion ratio of 6β-OHC/cortisol. Differences in the inhibitory activity on cytochrome *P*450-dependent enzymes observed between selected contraceptive steroids *in vitro* were not observed in the same way *in vivo*. This is either due to the absence of a marked inhibitory activity *in vivo* or

the extent of this activity is so small that the sensitivity of the marker 6β-OHC/cortisol is not sufficient to detect these changes. In this context, it is important to recall that a pronounced inhibitory activity was observed *in vitro* only at relatively high drug concentrations, which were well above those measured in women under oral contraceptive therapy.

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