THE EFFECT OF ETHINYL ESTRADIOL 20 mcg AND LEVONORGESTREL 250 mcg ON THE PITUITARY-OVARIAN FUNCTION DURING NORMAL TABLET-TAKING AND WHEN TABLETS ARE MISSED

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ABSTRACT

A new combined pill containing 20 micrograms of ethinyl estradiol and 250 micrograms of levonorgestrel has been developed. The safety margin of this type of low-dose preparation needed to be assessed and this was done by evaluating daily levels of LH, FSH, estradiol, progesterone, 1-NG and EE, as well as cervical mucus characteristics in six patients when one and then two consecutive pills were deliberately omitted mid-way through the cycle. Results demonstrated that there was no evidence of breakthrough ovulation, although there was some continued ovarian steroidogenesis, a feature consistent with previous studies using combined preparations. Existing instructions to patients regarding missed pills should continue in order to ensure maximal contraceptive safety.

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CONTRACEPTION

INTRODUCTION

In 1973 oestrogen dosage in some combined oral contraceptives dropped from 50 to 30 micrograms. One of the main worries to prescribing doctors was the margin of effectiveness of these new preparations. There was concern as to whether they efficiently suppressed ovulation and to their margin of error if tablets were missed. Extensive usage of these new products and clinical trials have shown that they are as reliable as their older counterparts containing 50 micrograms of oestrogen, in suppressing ovulation (1).

A previous study (2) indicated that omission of a pill containing 30 micrograms of ethinyl estradiol (EE₂) and 150 micrograms of levonorgestrel (l-NG) (Microgynon 30) early or late in the cycle was not associated with any reduced contraceptive action. An earlier study in which Microgynon was shown to have been omitted on day 12 of pill taking demonstrated that a sharp rise in gonadotrophins occurred, although cervical mucus continued to display those physical characteristics associated with an ancillary contraceptive effect (Schering information, on file). These studies concluded that further trials were necessary to assess the effect of increasing duration of pill omission on the pituitary-ovarian axis and cervical mucus, in order to evaluate the safety margin of these formulations.

This study evaluates a newer low-dose combination of 20 micrograms of EE₂ plus 250 micrograms l-NG (20/250 combination) which is undergoing clinical assessment at the present time. The tablet is sugar-coated and has been shown to have the same bioavailability as the plain tablet (Nuttall, I.D., personal communication). The aim of this study is to compare effects of this pill on the pituitary-ovarian axis and cervical mucus when tablets are taken normally and when they are missed.

METHODS

Six healthy female subjects of proven fertility were recruited. Their ages were between 18 and 40 and they had taken no hormonal preparation for 3 months prior to the commencement of the study. They had regular menstrual cycles. The study was open and continued for four cycles as follows:

Cycle 1 - Control cycle with no tablets taken.

Cycle 2 - All tablets of the 20/250 combination taken except for tablet number 10 (day 14 of the cycle).

Cycle 3 - All tablets of the 20/250 combination taken except for tablets 9 and 10 (days 13 and 14 of the cycle).

Cycle 4 - All tablets of the 20/250 combination taken.

The first tablet of the 20/250 combination was taken on the 5th day after the start of menstruation in cycle 2. The tablets were taken at the same pre-arranged time throughout the three cycles, i.e. 2 hours before blood sampling.
**CONTRACEPTION**

**Blood samples:** In the first and fourth cycles, 10ml of blood was taken at the same time each day for 21 days starting on the fifth day of the cycles. Second and third cycles - 10ml of blood was taken for eight days starting on the eleventh day of the cycle. The blood samples were stored at 4°C and allowed to clot. The serum was separated and allowed to stand for 24 hours in a refrigerator. After this time, the serum was decanted and deep frozen at -20°C in readiness for transportation to Edinburgh for analysis.

**Cervical mucus:** Cervical mucus was taken during the cycle at the same time as blood sampling on specified days each cycle. In cycle 1 specimens were taken on days 7, 9, 11, 12, 13, 14, 15, 17, 20 and 23. In cycle 2 specimens were taken on days 14, 15 and 16. In the fourth cycle specimens were taken on day 12 and day 20. The cervical mucus samples were examined for their physical characteristics and then stored at -20°C for biochemical analyses.

**Hormone assay methods:** Plasma l-norgestrel (l-NG), ethinyl estradiol (EE), progesterone, and estradiol were estimated by specific radioimmunoassays using 3H-labelled ligands, and dextran-coated charcoal for separation of free antibody-bound hormone. Intra-assay precision (coefficient of variation) was 7.8, 6.3, 7.8, and 11.5% for l-NG, EE, progesterone and estradiol, respectively. The corresponding values for inter-assay precision were 12.3, 12.4, 12.3 and 14.8%.

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) were estimated by methods based on those described by Hunter and Bennie (3). Intra-assay precision was 6.3 and 9.4% for FSH and LH, respectively; the corresponding values for inter-assay precision being 9.0 and 13.9%. Specimens from each complete cycle were analysed within a single batch for each hormone.

Results for FSH and LH were expressed in terms of standards provided by the National Institute of Biological Standards and Control (NIBSC), Holly Hill, London: the standards being designated 69/104 and 68/40 for FSH and LH, respectively. Reference values for hormone levels during the follicular phase are FSH: 3.0-15.0 U/l, LH: 2.5-9.0 U/l, progesterone: 2 nmol/l, and estradiol-17: 0.08-0.25 nmol/l. Normal luteal phase levels of progesterone and estradiol-17 are 22-88 nmol/l and 0.4-0.9 nmol/l, respectively.

**RESULTS**

**CYCLE 1 (control cycle, no medication) (Figs 1-6)**

Five women showed a normal follicular and luteal phase in the control cycles. The sixth patient (patient S) suggested that an anovulatory control cycle had occurred (Fig. 6).

**CYCLE 2 (1st pill cycle - pill missed on day 14 of the cycle)**

In cycle 2 blood samples were taken from day 11 to day 18 of the cycle (Fig. 7).
Figure 1. Plasma levels of FSH, LH, progesterone (prog), estradiol ($E_2$), 1-norgestrel (1-NG) and ethinyl estradiol (EE$_2$) in cycle 1 (control cycle) and cycle 4 (normal oral contraceptive medication).
Figure 2. Plasma levels of FSH, LH, progesterone (prog), estradiol ($E_2$), 1-norgestrel (1-NG) and ethinyl estradiol ($EE_2$) in cycle 1 (control cycle) and cycle 4 (normal oral contraceptive medication).
Figure 3. Plasma levels of FSH, LH, progesterone (prog), estradiol \((E_2)\), 1-norgestrel (1-NG) and ethinyl estradiol \((EE_2)\) in cycle 1 (control cycle) and cycle 4 (normal oral contraceptive medication).
Figure 4. Plasma levels of FSH, LH, progesterone (prog), estradiol (E₂), 1-norgestrel (1-NG) and ethinyl estradiol (EE₂) in cycle 1 (control cycle) and cycle 4 (normal oral contraceptive medication).
Figure 5. Plasma levels of FSH, LH, progesterone (prog), estradiol (E$_2$), 1-norgestrel (1-NG) and ethinyl estradiol (EE$_2$) in cycle 1 (control cycle) and cycle 4 (normal oral contraceptive medication).
Figure 6. Plasma levels of FSH, LH, progesterone (prog), estradiol (E₂), 1-norgestrel (1-NG) and ethinyl estradiol (EE₂) in cycle 1 (control cycle) and cycle 4 (normal oral contraceptive medication).
Pill missed on day 14

Figure 7. Plasma levels of FSH, LH, Prog, E\textsubscript{2}, 1-NG and EE\textsubscript{2} in cycle 2 in which one pill was missed on day 14.
Natural steroid hormones and gonadotrophins

Plasma estradiol and progesterone levels in all subjects were suppressed between days 11 and 18, and remained unchanged after one pill was missed. No peaks of FSH and LH were found, although subject Y showed increases in both FSH and LH 48 hours after pill omission and subject W showed a slight rise in LH.

Synthetic steroids

Both synthetic steroid values reflect the day when the pill was missed. Subject W appears to have missed her pill on day 13, as evidenced by her plasma synthetic steroid concentration on this day, but this could not be confirmed.

Bleeding patterns

One of the subjects, E, experienced a heavy blood loss on day 16, with slight blood losses on days 17 and 18, and a slight discharge on days 15, 19, 20 and 21. Another subject, G, experienced slight spotting on day 15. None of the other four subjects had any problems.

CYCLE 3 (2nd pill cycle - pill missed on days 13 and 14)

Blood samples were taken from day 11 to day 18 of the cycle (Fig. 8).

Natural steroid hormones and gonadotrophins

In all subjects, except W, estradiol and progesterone were suppressed although subjects E and Y showed very slight pulses of estradiol within 24 hours of pill omission. Estradiol and progesterone levels in subject W were raised in this cycle, but never to levels approaching ovulation values. She also showed the greatest LH, but no change in FSH values. Unfortunately, no collections were received for day 14 for subject E, who showed a slight surge of LH values on day 13. No corresponding FSH surge was noted. Subject G also showed a slight surge in LH and FSH values on day 14, but again, unfortunately no samples were taken on day 15.

Synthetic steroids

These again reflect the days on which the pill was omitted. Subject W appears to have missed her pill on days 12 and 13, and subject Y on days 14 and 15.

Bleeding patterns

Subject W experienced heavy bleeding on day 16, with spotting on days 14-21. Subject G also had bleeding problems on day 16, with lighter discharged on day 14 and 15. Subject Y had no bleeding problems, and both N and E had slight spotting problems on days 14-17 and day 15, respectively. No other problems were reported in this cycle.
Figure 8. Plasma levels of FSH, LH, Prog, E₂, 1-NG and EE in cycle 3 in which pills were missed on days 13 and 14.
It is interesting to note that subject E's bleeding problems were less severe after missing two tablets, than after missing one tablet, possibly reflecting that one month is needed to stabilise on this preparation. All six subjects reported normal periods of shorter duration throughout all three pill cycles.

**CYCLE 4 (complete pill control) (Figs. 1-6)**

Blood samples were collected from day 5 to day 25 of the cycle.

**Natural steroid hormones and gonadotrophins**

Estradiol, progesterone, FSH and LH are totally suppressed throughout the cycle in all six women, although E2, FSH and LH tend to remain marginally higher at the beginning of the cycle. No mid-cycle peaks for any of these hormones are evident.

**Synthetic steroids**

Both l-NG and EE2 also tend to stabilise within two or three days of pill taking, although large daily fluctuations within and between women are evident, especially for l-NG. Synthetic steroid levels in cycle 4 for individual subjects lay in the range 3.69-24.5 nmol/l/day for l-NG and 0.90-1.80 nmol/l/day for EE2.

**Bleeding patterns**

No bleeding problems were experienced by any subject in this cycle, nor were any adverse side effects noted.

**CERVICAL MUCUS**

Increased viscosity was noted in the samples obtained in the pill cycle, as opposed to the control cycles, when the characteristic rheological changes of an ovulatory cycle were observed. Biochemical analyses were not possible, however, owing to the loss of these specimens as a result of a deep-freeze failure.

**DISCUSSION**

A previous study (2) using Microgynon 30 has suggested that there was an adequate margin of effectiveness even when pills were missed on day 4, and on day 19 of pill taking. It was noted that estradiol levels were similar to those observed in a typical early follicular phase and in the occasional patient estradiol concentration reached mid-cycle level in a normal ovulatory cycle. This suggested that there was a degree of ovarian steroidogenesis and this feature has been noted in other studies of combined preparations (4). Despite this follicular activity, there was no evidence of ovulation as evidenced by other parameters during this missed pill study, confirming that this formulation had a fair margin of effectiveness. This was reinforced by a continued progestational cervical mucus.

However, the occurrence of a degree of ovarian steroidogenesis in these earlier trials suggested that it would be interesting to assess plasma hormone levels if more than one pill was missed consecutively.
A new formulation of 20 micrograms of EE and 250 micrograms of l-NG is at present undergoing clinical trial. Previous studies suggested that it is an effective inhibitor of ovulation and maintains good cycle control (Schering information, on file). It was therefore considered appropriate to see whether this preparation maintained the same margin of effectiveness when pills were deliberately missed. In this study the deliberate pill omission occurred on days 14 and 14/15 in order to assess whether a surge of estradiol would occur at this significant time of the cycle thereby stimulating a peak of LH by positive feedback.

The results of this study suggested that this preparation was effective in suppressing ovulation even when two pills were missed on consecutive days mid-way in their course of administration. In no cases did the plasma hormone levels reach ovulatory levels. However, in some patients following pill omission, there were small peaks of FSH and LH and, as has been noted in a previous study (2), small pulses of estradiol and FSH occurred within 24 hours of pill omission. This confirmed that in cases of pill omission a small degree of ovarian steroidogenesis may be occurring but never to levels approaching ovulation values. It must be remembered that in patient 5 the control cycle was anovulatory and therefore her results were unhelpful. However, it was clear that she did have some ovarian activity in the control cycle as shown by the raised estradiol levels, and these levels were completely suppressed in all her pill-taking cycles.

The levels of synthetic steroids reflected the days on which pills were missed but it was clear from these results that on occasion patients did miss the pills on the wrong days. In the pill-taking cycle where no pills were missed (cycle 4), it was noticed that the levels of synthetic steroids stabilised within two or three days of pill taking but there were large daily fluctuations within and between the subjects particularly for the levels of l-NG. These results confirm previous bioavailability studies (Schering information, on file) which suggest large physiological variation between patients in the pharmacokinetics of the synthetic steroid hormones. Studies of the bleeding pattern confirmed that in some subjects intermenstrual spotting will occur when pills are missed even though this does not necessarily mean that ovulation has occurred as evidenced by the plasma hormone levels. However, intermenstrual bleeding occurring after previous regular cycles on the pill may suggest the patient has missed some tablets or that there is an interaction with another drug.

Unfortunately, due to a laboratory accident, many cervical mucus samples taken during this study were ruined. This prevented a comprehensive biochemical assessment of the cervical mucus. However, when each sample was taken, an assessment of its physical characteristics was made, which confirmed that during all the pill-taking cycles the cervical mucus had a thick viscous character which was not present during the control cycles. This thick viscous characteristic was not altered during the pill omission cycles.

In conclusion, this study demonstrated the fairly wide margin of effectiveness that this combined preparation possesses. However, the recommendations made to pill takers regarding pill omission should
stand to ensure a maximum effectiveness of these preparations. Thus, when one or even two tablets are omitted in the latter half of the cycle, extra contraceptive protection would not appear to be required, even if breakthrough bleeding occurs. Our experimental data however do not allow us to extend this to earlier in the cycle or for more omissions of tablet taking than the two described. More studies along these lines are required.

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REFERENCES


