ANDROGENIC, ANABOLIC, ESTROGENIC AND ANTIESTROGENIC EFFECTS OF DESOGESTREL AND LYNESTRENOL: EFFECTS ON SERUM PROTEINS AND VAGINAL CYTOLOGY

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ABSTRACT

Eight healthy (apart from pelvic endometriosis) women were given daily doses of 0.125, 0.250 and 0.500 mg of desogestrel or 5 mg of lynestrenol orally in a randomized order. Duration of each treatment was 6 weeks. Serum was analyzed for sex hormone binding globulin (SHBG), ceruloplasmin, cortisol binding globulin (CBG), thyroxine binding globulin (TBG) and prealbumin using an electroimmunoassay. Serum 17β-estradiol (E_2) and testosterone (T) were analyzed by radioimmunoassay. Vaginal cytology was studied using the maturation value (MV).

E_2 levels were depressed by desogestrel and lynestrenol apart from values in two women after 0.125 mg desogestrel. T concentration was suppressed by desogestrel but not by lynestrenol.

SHBG concentration and MV were dose-dependently suppressed indicating an antiestrogenic or possibly androgenic effect of desogestrel and lynestrenol. No androgenic or anabolic effects of desogestrel were however seen, e.g. suppression of TBG content or increase in prealbumin levels. For lynestrenol, however, a small but significant increase in prealbumin concentration indicated a weak androgenic/anabolic effect. No estrogenic effects were seen, e.g. increases in ceruloplasmin, CBG levels or in elevations of MV.

A depressed SHBG production ability in the hepatocytes during treatment with 19-nortestosterone derivatives is postulated, possibly due to competitive receptor binding.

INTRODUCTION

A new progestational compound, desogestrel (17-α-ethynyl-18-methyl-11-methylene-4-estrene-17β-ol) has been developed by Organon, the Netherlands. Desogestrel is a gonane derivate of 19-nortestosterone; the parent tetracyclic hydrocarbon is without methyl groups at C_{10} and C_{13} and has no side-chain at C_{17}. It has structural similarities (Fig 1) to the estrane lynestrenol (an estrane has a methyl group at C_{13} but is otherwise the same as a gonane).

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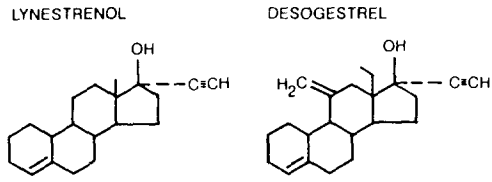

Figure 1. Structure formulas for lynestrenol and desogestrel.

Desogestrel has been shown in animal experiments to be a strong progestational compound (1) with no estrogenic and very weak androgenic effects; the antiestrogenicity evaluated as the potency to counteract estrogenic effects on vaginal cytology was intermediate. The progestational activity has also been demonstrated in humans (2, 3). Other endocrinological properties such as androgenicity or estrogenicity are of great clinical importance but are difficult to demonstrate conclusively in humans. However, some serum proteins are influenced by sex hormones in typical patterns dependent of the properties of the hormones (4, 5, 6, 7, 8), e.g. increased levels are seen in ceruloplasmin, cortisol binding globulin (CBG) and sex hormone binding globulin (SHBG) after estrogens and in prealbumin after androgens, for prealbumin partly in relation to their anabolic effect. SHBG and thyroxin binding globulin (TBG) levels are lowered by androgens, TBG especially by those with anabolic effects.

When desogestrel was given alone in small doses (0.03 mg) to healthy women, no effect was observed on estrogen-sensitive ceruloplasmin (3). However, when administered in combination with 0.03 mg ethinylestradiol (EE) (4, 9), there was a pronounced increase in the estrogen-androgen sensitive sex hormone binding globulin (SHBG) corresponding to the effect of EE alone (9). This unopposed increase in SHBG by EE may represent an estrogenicity of desogestrel or a lack of anti-estrogenic or androgenic effects (4). However, Crona *et al.* (9) showed that desogestrel, as well as levonorgestrel alone, depressed the levels of SHBG and considered the changes to be due to androgenic effects. In order to study the alternatives of estrogenicity, antiestrogenicity and androgenicity, the present study was performed to analyze the effects of desogestrel and lynestrenol alone in several sex hormone-sensitive plasma proteins, some endogenous sex hormones and vaginal cytology.

METHODS AND MATERIAL

Eight women with regular menstrual cycles, healthy apart from laparoscopy-verified endometriosis, agreed to take part in the study and gave informed consent. The patients' mean age was 28.5 years with a range of 21 - 35. They had all been without any hormonal medication for at least four months.

All women received 0.125, 0.250 and 0.500 mg of desogestrel and 5 mg of lynestrenol, in a randomized order. All treatment periods were six weeks without intervals between the treatment periods and the first tablet was taken on the first day of a menstrual cycle.

Before treatment blood was drawn around day 7 - 9 in the cycle; another blood sample was then obtained at each visit every six weeks. Blood was centrifuged and serum was kept frozen at – 26°C until analyzed.

Serum testosterone and 17β-estradiol (E_2) were analyzed by radioimmunoassay as described earlier (3, 4). Serum was further analyzed for SHBG, thyroxine binding globulin (TBG), ceruloplasmin, cortisol binding globulin (CBG) and prealbumin by electroimmunoassay as described by Laurell and Rannevik (5).

Vaginal cytology was judged on samples taken from the lateral vaginal wall. They were stained according to Papanicolau and 200 cells were counted to establish a maturation index. This index was then broken down to give a maturation value (10) (MV) by adding the percentage of intermediate cells multiplied by a factor 0.5 to the percentage of superficial cells. The parabasal cell percentage was not included. Sampling was performed before treatment and after each treatment period.

Statistics used was a covariance analysis to show dose-response relationships. Contrasts among specific means were also tested. Correlations were analyzed with the least square method and significances of correlation were tested with a student's t test.

RESULTS

All form of treatments resulted in some intermenstrual bleedings and spottings; there was no difference between the various treatments. No other side effects of clinical importance as weight gain, acne or hirsutism were observed.

Pretreatment E_2 levels per individual were highly variable (Fig 2).

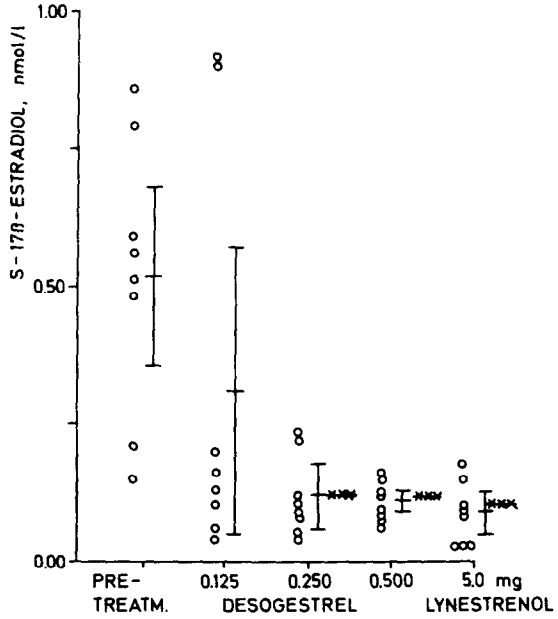

*Figure 2. Individual serum levels of 17β-estradiol in 8 fertile women before treatment on cycle day 7 - 9 and after 6 weeks of treatment with 0.125, 0.250 and 0.500 mg desogestrel and 5 mg lynestrenol. Treatments were given in a randomized order without intervals. Vertical bars represent means and ± 2 SEM. Statistical significances between pretreatment and treatment values are indicated with asterisks: *** = P < 0.001.*

During treatment the serum E_2 levels were depressed ($P < 0.001$) dose-dependently except for 0.125 mg desogestrel. During administration of this dose, six women decreased in E_2 levels while two had higher values of E_2 after treatment than before. The depression achieved with 5 mg lynestrenol corresponded to about 0.6 mg desogestrel.

Serum testosterone levels in nmol/l were depressed ($P < 0.001$) from 2.1 ± 0.10 (mean ± SEM) to 1.8 ± 0.03, 1.7 ± 0.05 and 1.4 ± 0.10 with increasing desogestrel doses. During lynestrenol treatment a small but insignificant depression of T was observed (from 2.1 ± 0.10 to 1.9 ± 0.10). No correlations were shown with any of the serum proteins or with MV.

SHBG levels (Fig 3, left panel) were within the normal range (50 - 150%) before treatment. They were lowered during treatment also in the two patients with high levels of 17β-estradiol on the 0.125 mg desogestrel dose. However, two other patients showed increases in SHBG levels rendering the decrease statistically insignificant at this dose level. For 0.25 and 0.50 mg desogestrel the depression was significant ($P < 0.001$) (Fig 3, left panel). The correlation SHBG - E_2 (Fig 3, right panel) was significant ($R = 0.54$, $P < 0.05$) but much weaker than the correlation between the dose of desogestrel and SHBG ($R = 0.97$, $P < 0.001$).

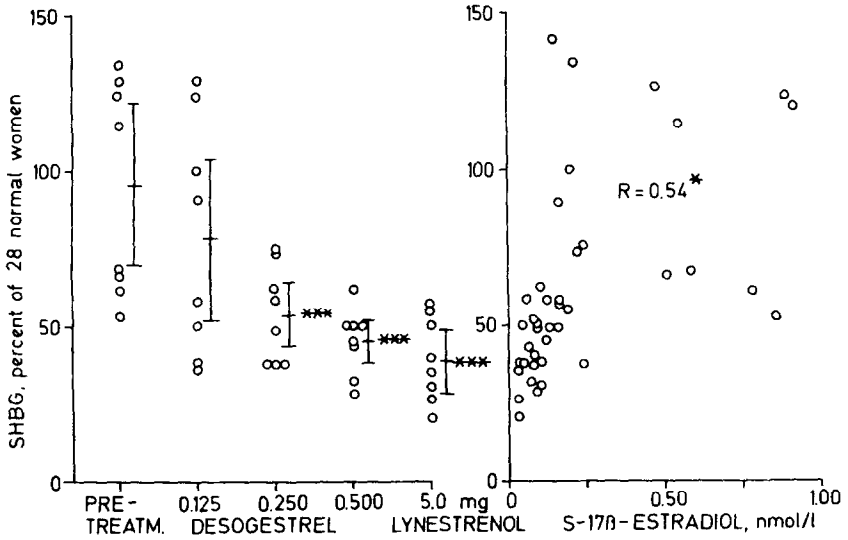

Figure 3. ***Left panel:*** *Individual levels of S - SHBG determined by electroimmunoassay. Levels are given as percentages of a reference pool of serum from 28 untreated healthy fertile women. Volunteers, treatments and symbols as in Fig 2.* ***Right panel:*** *Correlation between SHBG and 17β-estradiol with pretreatment and treatment values given in Fig 2 and 3, left panel. R = correlation coefficient. * = significant correlation, $P < 0.05$.*

The effect of 5 mg lynestrenol corresponded to 0.5 mg desogestrel.

Serum ceruloplasmin, CBG and TBG were not significantly influenced by the treatments.

Serum prealbumin was not influenced by desogestrel but lynestrenol induced an increase ($P < 0.01$) from 0.29 ± 0.01 g/l (mean ± SEM) to 0.34 ± 0.02.

Maturation value (Fig 4) was significantly depressed by desogestrel, the decrease being dose-dependent. The lowest dose gave a strong effect ($P < 0.001$) in spite of increased levels of E_2 in two women and further decrease in MV was insignificant. The lynestrenol effect was extrapolated to equal about 0.6 mg desogestrel. The correlation between E_2 and MV was high ($R = 0.84$, $P < 0.001$) in the total material but no correlation was found between MV and dose of desogestrel.

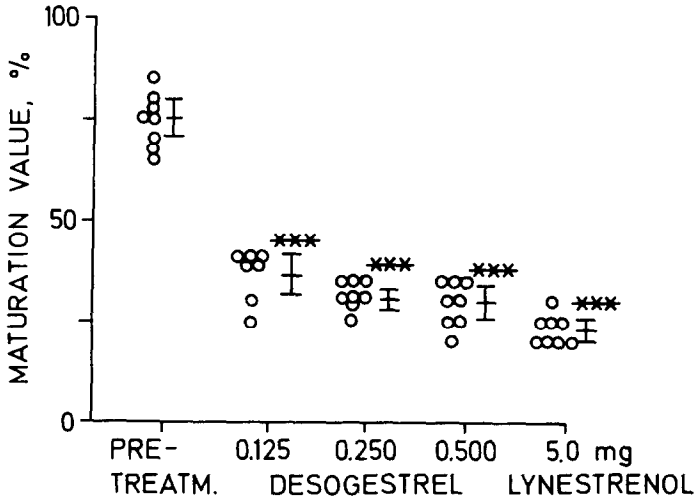

Figure 4. Individual maturation values in percent. Volunteers, treatments and symbols are the same as in Fig 2.

DISCUSSION

In the present study desogestrel and lynestrenol were given in doses well above the ovulation inhibiting dose of 0.06 and 0.6 - 0.7 mg, respectively (3). A depression in E_2 and T production after higher doses is to be expected due to hypothalamic inhibition of the gonadotropin-releasing hormone and was also seen in this study. Sometimes this inhibition is not complete and high E_2 values can be found as shown in studies on continuous low-dose progestogen administration (3, 12). Two such high E_2 values were found in spite of 0.125 mg desogestrel but then a progressive dose-dependent lowering of E_2 was present.

SHBG levels are increased by estrogens and depressed by androgens (5, 6). The threshold for E_2 to induce SHBG production is high while even small doses of synthetic estrogens as EE gives strong increases. The low E_2 values could be taken as the cause for the lowering of SHBG seen in this study. However, anovulatory or postmenopausal women with low E_2 values have almost normal levels of estrogen-sensitive serum proteins as SHBG, ceruloplasmin, CBG and TBG (5).

The decrease of SHBG is apparently not a progestational effect since ovulation inhibiting and E_2-depressing doses of medroxyprogesterone acetate administered vaginally and resulting in serum levels comparable to oral dose of 10 mg/24 h had no effect on SHBG (13). Megestrol acetate alone even induces an increase in SHBG levels (7). Both these substances are, however, 17*a*-hydroxyprogesterone derivatives which appear to lack antiestrogenic and androgenic effects. In contrast levonorgestrel as a 19-nortestosterone-derivative depresses SHBG significantly when given alone in low (0.075 mg) (7) to moderate (0.15 mg) (9) doses. Briggs, however, found only nonsignificant SHBG decreases after 0.25 mg levonorgestrel and 1 mg lynestrenol orally (14) while 0.2 mg/24 h by the vaginal route induced significant decrease in SGHB levels (13). The drop in SHBG with the 19-nortestosterone-derivatives could be ascribed to an androgenic effect. An androgenic effect would also lead to increased levels of prealbumin (5, 6) and lowered levels of TBG (5, 6). There are thus no signs of androgenic effects after treatment with desogestrel judging from the lack of changes in prealbumin and TBG in the present study and, when combined with EE (4), the pronounced increasing effect on SHBG.

Changes in prealbumin and TBG are also related to the anabolic effects (*i.e.* nitrogen retaining »myotropic» effects) of a hormonal compound (5, 6) and no such effect is thus seen in desogestrel. The depressing effect on SHBG by lynestrenol may, however, be due to a weak anabolic/androgenic activity as seen in a small but significant increase in prealbumin in the present study and in blocking of SHBG increases in combintaion with EE (15). The mechanism of this SHBG production blocking effect is not clear. Desogestrel has a very low affinity for the estrogen receptor (16) as has levonorgestrel and the binding to SHBG for desogestrel and its main metabolite 3-keto-desogestrel is much weaker than that of levonorgestrel (16) and of testosterone (13, 16). It is thus not very probable that displacement of testosterone from SHBG by desogestrel may play any role as has been discussed for levonorgestrel (7, 13, 17). Total testosterone values were also lowered by desogestrel and not indicating a SHBG-depressing mechanism analogous to that in PCO women (11). However, with lowered SHBG, high free testosterone levels may follow (17) but no androgenic side effects of treatment (weight gain, hirsutism or acne) were seen in the present study. A theory was discussed by Rannevik and Laurell concerning the 17*a*-alkylated progestational compound danazol (5): Hepatocyte estrogen receptors are competitively blocked by inactivation of the corresponding chromatin template. This still remains to be proven probably by *in vitro* assays on liver tissues.

Signs of antiestrogenicity for desogestrel may be found in vaginal cytology as in lowering of the maturation value even after 0.125 mg desogestrel in spite of, in two cases, adequate E_2 levels. Higher desogestrel doses gave only a moderate further MV depression.

Briggs (18) showed that levonorgestrel could counteract the EE-induced increase in ceruloplasmin levels by an antiestrogenic effect. Several other studies have, however, not been able to reproduce this finding (4, 5, 9). The lack of changes in either direction with desogestrel and lynestrenol in the present study thus confirms the findings in animal pharmacology (1) that desogestrel and lynestrenol have no estrogenic effects.

CONCLUSION

In conclusion, no estrogenicity can be detected in desogestrel or lynestrenol from serum proteins or vaginal cytology. The lack of effect on ceruloplasmin, cortisol binding globulin and thyroxine binding globulin may be strong evidences since the sensitivity of these proteins for synthetic estrogens is high (4).

Signs of antiestrogenic properties are noted in both SHBG and vaginal cytology since, in spite of adequate E_2 levels, SHBG levels are lowered as well as MV. There are no anabolic or androgenic effects in desogestrel since there is no effect on TBG or prealbumin. Desogestrel is about 10 times as potent per milligram compared to lynestrenol based on the effects on serum proteins.

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REFERENCES

1. de Visser J, de Jager E, de Jongh H, van der Vies J, Zeelen F: Pharmacological profile of a new orally active progestational steroid, Org 2969. Acta Endocrinol (Kbh) Suppl 199:405, 1975
2. Cullberg G: Org 2969, a new progestational compound. Reproduccion 2:333, 1975
3. Cullberg G, Lindstedt G, Lundberg P-A, Steffensen K: Central and peripheral effects of desogestrel 15-60 μg daily for 21 days in healthy female volunteers. Acta Obstet Gynecol Scand Suppl 111:21, 1982
4. Cullberg G, Dovre P-A, Lindstedt G, Steffensen K: On the use of plasma proteins as indicators of the metabolic effects of combined oral contraceptives. Acta Obstet Gynecol Scand Suppl 111:47, 1982
5. Laurell C-B, Rannevik G: A comparison of plasma protein changes induced by danazol, pregnancy and oestrogens. J Clin Endocrinol Metab 49:719, 1979
6. Barbosa J, Seal US, Doe RP: Effect of anabolic steroids on hormone-binding proteins, serum cortisol and serum nonprotein-bound cortisol. J Clin Endocrinol 32:232, 1971
7. el Makhzangy MN, Wynn V, Lawrence DM: Sex hormone binding globulin capacity as an index of oestrogenicity or androgenicity in women on oral contraceptive steroids. Clin Endocrinol 10:39, 1979
8. Schwartz U, Hammerstein J: The oestrogenic potency of various contraceptive steroids as determined by their effects on transcortin-binding capacity. Acta Endocrinol (Kbh) 76:159, 1974
9. Crona N, Silverstolpe G, Samsioe G: Changes in serum apo-lipoprotein A I and sex hormone-binding globulin levels after treatment with two different progestins administered alone and in combination with ethinylestradiol. Diss. University of Gothenburg, p V:1, 1983
10. Meisels A: The maturation value. Acta Cytol 11: 246, 1967
11. Lobo RA, Goebelsman U: Effect of androgen excess on inappropriate gonadotropin secretion as found in the polycystic ovary syndrome. Am J Obstet Gynecol 142:394, 1982
12. Johansson EDB: Effects of exogenous steroids on gonadotropin secretion. Clin Obstet Gynecol 3:579, 1976
13. Victor A, Johansson EDB: Effects of d-norgestrel-induced decreases in sex hormone binding globulin capacity on the d-norgestrel levels in plasma. Contraception 16 (2):115, 1977
14. Briggs MH: Hormonal contraceptives and plasma sex hormone binding globulin. Contraception 12:149, 1975
15. Odlind V, Weiner E, Victor A, Johansson EDB: Effects on sex hormone binding globulin of different oral contraceptives containing norethisterone and lynestrenol. Brit J Obstet Gynecol 17:416, 1980
16. Bergink EW, Hamburger AD, de Jager E, van der Vies J: Binding of a contraceptive progestogen Org 2969 and its metabolites to receptor proteins and human sex hormone binding globulin. J Ster Biochem 14:175, 1981
17. Stumpf PG, Nakamura RM, Mishell DR jr: Changes in physiologically free circulating estradiol and testosterone during exposure to levonorgestrel. J Clin Endocrin Metab 52 (1):138, 1981
18. Briggs M: Effect of oral progestogens on estrogen-induced changes in serum protein. In: The Second International Norgestrel Symposium (ed. D.V.I. Fairweather). Excerpta Medica Int Congr Ser 344:35, 1974