Danazol has progestin-like actions on the human endometrium

E. Kokko, O. Jänne, A. Kauppila,
L. Rönberg and R. Vihko

Departments of Clinical Chemistry, Obstetrics and Gynaecology, and Biochemistry,
University of Oulu, SF-90220 Oulu 22, Finland

Abstract. The administration of danazol, 200 mg three times daily, from the 3rd to the 23rd day of the cycle to normally menstruating women exhibited the following actions on the human endometrium: significantly reduced cytosol oestrogen and progestin receptor concentrations, and declined 17β-hydroxysteroid dehydrogenase activity. Very similar results were obtained during medroxyprogesterone acetate (100 mg daily) treatment for the same period of time. Danazol administration did not decrease circulating gonadotrophin levels but clearly suppressed luteal serum oestradiol and progesterone concentrations. Danazol was found to bind in vitro to endometrial progestin receptor with an affinity approximately 3% of that of progesterone. These findings are compatible with the notion that a local progestin-like rather than a systemic action of danazol is the way by which its therapeutic effect is exerted. This may be potentiated by the suppression of circulating oestradiol levels.

Danazol is widely used in the treatment of endometriosis and it is increasingly applied in the management of other gynaecological disorders (Dmowski 1979; Jenkin 1979). The mechanism of action of danazol is poorly understood. Danazol was shown to suppress elevated gonadotrophin levels in postmenopausal women, but this antigonadotrophic effect is not clear in a number of physiological and experimental conditions (Jenkin 1979). Danazol has also been reported to interact with a number of steroid receptors (Barbieri et al. 1979; Channess et al. 1980), and to exert inhibitory effects on gonadal steroidogenesis (Barbieri et al. 1977a,b). The minor interaction of danazol with progestin receptor

has been interpreted as an antiprogestational property of danazol (Jenkin 1979).

To extend our studies on the mechanism of progestin action in human endometrium (Jänne et al. 1979; Vihko et al. 1980), we investigated the effects of a 3 week treatment period of danazol on cytosol and nuclear oestrogen (ER) and progestin (PR) receptors, and on the activity of 17β-hydroxysteroid dehydrogenase (17β-HSD) in the endometrium of normally cycling women. The effects observed were very similar to those of medroxyprogesterone acetate (MPA) administration. In addition, the effects on serum gonadotrophin, prolactin, oestradiol and progesterone concentrations were investigated.

Material and Methods

Chemicals

Tritium-labelled [2,4,6,7-3H]oestradiol1 (S.A. 101 Ci/mmol), [6,7-3H]ORG 2058 (S.A. 58 Ci/mmol) and [4-14C]oestrone (S.A. 58 Ci/mmol) and non-labelled ORG 2058 were purchased from The Radiochemical

1 Trivial and systematic names of steroids:

Oestradiol: 1,3,5(10)-oestratriene-3,17β-diol.
Oestrone: 3-hydroxy-1,3,5(10)-oestratrien-17-one.
Medroxyprogesterone acetate: 6α-methyl-17α-hydroxy-19-nor-4-pregnene-3,20-dione acetate.
Progesterone: 4-pregnene-3,20-dione.
Testosterone: 17β-hydroxy-4-androsten-3-one.
Centre, Amersham, U.K. Non-labelled oestradiol, oestrone and testosterone were obtained from Steraloids Inc., Wilton, NH. Activated charcoal (Norit A) was from Sigma, St. Louis, MO and dithiothreitol was purchased from Calbiochem, San Diego, CA. Other chemicals were from Merck AG., Darmstadt, FRG, and were of the highest purity grade available.

**Subjects**

Nine voluntary and well informed normally menstruating women were administered danazol, 200 mg thrice daily, from the 3rd to the 23rd day of the cycle. Endometrial biopsies from the fundal part of the uterus were taken on the 23rd to 25th (usually 24th) day, one day after termination of the treatment. Endometrial samples were also taken from 40 non-treated women, and from 8 subjects receiving 100 mg of MPA. The specimens were immediately placed into small plastic bags, frozen in liquid nitrogen, and stored at −70°C until assayed. Blood samples were drawn from 4 women on danazol therapy, 8 women on MPA therapy and 8 controls on the 6th, 14th and 24th day between 08.00–10.00 h. Serum was separated by low speed centrifugation, divided into small aliquots, and stored at −20°C until assayed.

**Determination of serum hormone concentrations**

The serum levels of FSH, LH and prolactin were determined using CEA-1RE-SORIN (GIS) kits (Departments des Radioéléments, PB No. 21, F-91190 Gif-sur-Yvette, France), and following the manufacturer’s instructions and the standardization of these assays in our laboratory (Hammond et al. 1977a). All these assays included low, medium and high concentration control sera among the analytical samples.

The concentrations of oestradiol and progesterone were measured by radioimmunoassay using kits kindly donated by Nordiclab Oy, Oulu, Finland, following the manufacturer’s instructions and adopting a semi-automated processing of the tubes (Hammond et al. 1977b). Low and high concentration control sera were run in parallel with the samples.

**Processing of tissue samples**

Tissue samples were washed with ice-cold buffer TEND-10G (10 mM Tris-HCl, pH 7.5 at 23°C, 1.5 mM EDTA, 3 mM NaNO₃, 2 mM dithiothreitol, and 10%, v/v, glycerol), cut with scissors into small pieces and then homogenized in the TEND-10G buffer (4–6 ml/g tissue wet weight). All procedures were carried out at 0–4°C. The homogenate was centrifuged at 800 × g for 15 min, and the supernatant was divided into three parts. The first part was used for the determination of protein content, the second for the immediate determination of 17β-hydroxysteroid dehydrogenase and the third was centrifuged at 105 000 × g for 60 min to get the cytosol fraction, which was used for the determination of oestrogen and progesterin receptors.

The 800 g-sediment was re-homogenized twice in TEND-10G buffer (8 ml/g tissue wet weight) containing 1 mM MgCl₂ using a motor-driven Teflon-glass homogenizer with intermittent centrifugation at 800 × g for 15 min. The suspension after the third homogenization was used for determination of DNA, and nuclear oestrogen and progesterin receptor levels.

**Measurement of 17β-hydroxysteroid dehydrogenase activity**

17β-Hydroxysteroid dehydrogenase activity was measured as described by Tseng & Gurpide (1975). One ml of the 800 g-supernatant (protein content about 1 mg/ml) in TEND-10G buffer containing NAD⁺ (1.4 μmol/l) and [3H]oestradiol (3 × 10⁵ CPM, 37 nmol/l) was incubated for 20 min at 37°C, after which the tubes were transferred into ice-water bath. [14C]Oestrone, 0.010 ml, in ethanol was added as internal standard and the mixture was extracted twice with 2.0 ml ether. The combined ether phases were evaporated to dryness under nitrogen and dissolved in ethanol (0.1 ml). After Silica gel thin layer chromatography (solvent system: toluene-methanol, 92:8, v/v) the recovery of [3H]oestradiol was estimated using [14C]oestrone as indicator. The enzyme activity was calculated for the conversion of oestradiol to oestrone (nmol of oestrone formed/mg protein per 20 min).

**Determination of cytosol steroid receptor concentrations**

Tritiated oestradiol and ORG 2058 were used as labelled ligands in oestrogen and progesterin receptor assays, respectively, which were carried out as previously described (Kokko et al. 1981). The oestrogen receptor concentration was measured incubating 0.1 ml of cytosol (105 000 g-supernatant) with seven different concentrations (0.08–6.9 nM; 0.1 ml) of [3H]oestradiol in buffer TEND-10G for 18 h at 0–4°C followed by 1 h at 30°C for the measurement of total receptor concentration. Non-specific binding was estimated from parallel sets of tubes containing 200-fold molar excess of non-radioactive oestradiol. A 100-fold molar excess of testosterone was used to minimize the binding of oestradiol to plasma sex steroid binding globulin. After incubation the tubes were chilled in an ice-water bath for 15 min, and bound and free steroids were separated with dextran-charcoal. Receptor bound fraction of ligand was measured after centrifugation from the supernatant by liquid scintillation counting.

For the determination of cytosol progesterin receptors, 0.1 ml of cytosol was incubated with various concentrations of [3H]ORG 2058 (final concentrations: 0.16–11.2 nmol/l). This synthetic progesterin does not bind to any significant extent to plasma proteins (Kontula et al. 1973) and therefore no correction for the interference of plasma contaminants was necessary. A 200-fold molar excess of non-labelled ORG 2058 was used to determine the non-specific binding. The tubes were incubated for...
18 h at 4°C, and the following procedures were performed as outlined above for the oestrogen receptor.

The method of Scatchard (1949) was used for the calculation of cytosol oestrogen and progestin receptor levels, which were expressed as fmoles per mg cytosol protein.

Measurement of nuclear oestrogen and progestin receptors

The exchange technique described by Hsueh et al. (1974) was used in these assays. The suspension for nuclear receptor assay (0.5 ml) was incubated with 5 nM [3H]oestradiol and 500 nM testosterone for oestrogen receptors or 20 nM [3H]ORG 2058 for progestin receptors for 18 h at 0–4°C. A parallel set of tubes containing 200-fold excess of non-labelled oestradiol or ORG 2058, respectively, was incubated to monitor the non-specific binding of radioactivity. The tubes for oestrogen receptor assay were incubated an additional 1 h at 30°C to measure total nuclear oestrogen receptor level.

After the incubations the tubes were transferred into ice-water bath, 2 ml of ice-cold TEND-10G buffer containing 1.5 mM MgCl₂ was added, and the tubes were centrifuged at 2000 × g for 15 min at 0–4°C. After repeating this washing procedure twice the nuclear receptor bound ligand was extracted with ethanol, and the radioactivity was counted by liquid scintillation counting. The nuclear receptor level was calculated using an average DNA content of 6.0 pg/human endometrial cell.

Protein and DNA measurements

Protein concentrations were measured using the method of Lowry et al. (1951) with bovine serum albumin as standard. DNA was determined by the method of Burton (1956) with calf thymus DNA as standard.

Statistical analysis

Statistical analyses were performed with Student’s t-test (two-tailed, t-independent).

Results

Danazol bound in vitro to the cytosol PR of human endometrium with an affinity which was about 3% of that of progesterone. Its administration to normally cycling women significantly decreased cytosol ER concentration, and MPA treatment tended to

---

**Fig. 1.**

Cytosol and nuclear oestrogen (ER<sub>C</sub>, ER<sub>N</sub>) and progestin receptor (PR<sub>C</sub>, PR<sub>N</sub>) concentrations and 17β-hydroxysteroid dehydrogenase (17β-HSD) activity in the human endometrium of the control subjects (C) and of women treated with danazol (D) or medroxyprogesterone acetate (M). Both control and treatment samples were taken on the 24th day of the menstrual cycle. Asterisks indicate significantly lower than control values (* P < 0.05; ** P < 0.01; *** P < 0.001).
have a similar effect (Fig. 1). Both treatments decreased cytosol PR content (P < 0.001) and 17β-HSD activity (P < 0.05), but they did not have any significant effect on the nuclear ER and PR levels.

In the normally cycling women investigated, danazol did not significantly decrease serum concentrations of FSH, LH and prolactin measured on the different days of the cycle (Table 1). By contrast, there was a small but significant increase (P < 0.05) in the concentration of LH on day 6 of the cycle (Table 1). A decrease in serum oestradiol was significant in samples taken close to the end of the cycle. Danazol treatment also tended to decrease serum progesterone concentrations on day 24 of the cycle (Table 1).

The only changes in serum peptide hormone concentrations observed during MPA administration were the significant decreases in serum LH levels on the 14th and 24th days of the treatment cycle (Table 1). MPA also caused significant decreases in progesterone concentrations on the same day of the cycle.

Discussion

In the present study, the effects of a 3 week treatment with danazol or MPA on cytosol and nuclear ER and PR, and on the activity of 17β-HSD in the endometrium of normally cycling women were compared. A typical progestin effect, decreased cytosol PR content (Milgrom et al. 1973; Jänne et al. 1978), was seen after both danazol and MPA administration. Significantly decreased cytosol ER concentration seen after danazol treatment has also been observed in patients treated with MPA for endometrial hyperplasia or carcinoma (Jänne et al. 1979).

Danazol or MPA treatments did not significantly alter nuclear ER and PR levels. A significant decrease in the endometrial 17β-HSD activity was associated with the long-term administration of both danazol and MPA (Fig. 1). This effect is in sharp contrast to the more acute effects of progestrone leading to enhanced 17β-HSD activity in the endometrium (Tseng & Gurpide 1975), but it is possible that the ultimate biological action of progestins given for a long time is to terminate rather than maintain an acute progestin effect on the endometrium. Danazol seems also to have an acute progestin-like effect, as it induced early secretory changes in the endometrium of an ovariectomized woman on cyclic stilboestrol therapy (Wentz et al. 1976).

That danazol really has progestosterone-like properties was further supported by experiments in

---

Table 1.

Serum concentrations of FSH, LH, prolactin, oestradiol and progesterone in normal women and subjects receiving danazol and medroxyprogesterone acetate (MPA). The values are expressed as means ± SEM for the number of subjects in parentheses.

<table>
<thead>
<tr>
<th>Day of cycle</th>
<th>Treatment</th>
<th>FSH IU/l</th>
<th>LH IU/l</th>
<th>Prolactin µg/l</th>
<th>Oestradiol nmol/l</th>
<th>Progesterone nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>None</td>
<td>8.15 ± 0.82 (8)</td>
<td>8.23 ± 0.10 (8)</td>
<td>3.96 ± 0.73 (8)</td>
<td>0.13 ± 0.03 (8)</td>
<td>0.76 ± 0.43 (8)</td>
</tr>
<tr>
<td></td>
<td>Danazol</td>
<td>7.98 ± 0.33 (4)</td>
<td>9.90 ± 0.97* (4)</td>
<td>7.70 ± 4.08 (4)</td>
<td>0.09 ± 0.04 (4)</td>
<td>1.85 ± 0.34 (4)</td>
</tr>
<tr>
<td></td>
<td>MPA</td>
<td>9.98 ± 2.38 (7)</td>
<td>7.66 ± 0.93 (7)</td>
<td>4.24 ± 0.67 (7)</td>
<td>0.07 ± 0.02 (7)</td>
<td>0.95 ± 0.19 (6)</td>
</tr>
<tr>
<td>14</td>
<td>None</td>
<td>8.01 ± 1.32 (8)</td>
<td>16.70 ± 4.40 (8)</td>
<td>4.63 ± 0.94 (8)</td>
<td>0.50 ± 0.17 (8)</td>
<td>1.29 ± 0.14 (8)</td>
</tr>
<tr>
<td></td>
<td>Danazol</td>
<td>8.75 ± 2.20 (4)</td>
<td>16.45 ± 7.75 (4)</td>
<td>5.10 ± 2.07 (4)</td>
<td>0.37 ± 0.18 (4)</td>
<td>1.98 ± 0.39 (4)</td>
</tr>
<tr>
<td></td>
<td>MPA</td>
<td>6.16 ± 0.89 (7)</td>
<td>6.11 ± 0.78* (7)</td>
<td>4.74 ± 0.81 (7)</td>
<td>0.26 ± 0.17 (7)</td>
<td>0.70 ± 0.10** (6)</td>
</tr>
<tr>
<td>24</td>
<td>None</td>
<td>5.38 ± 0.61 (8)</td>
<td>13.00 ± 2.49 (8)</td>
<td>6.99 ± 1.72 (8)</td>
<td>0.36 ± 0.07 (8)</td>
<td>40.76 ± 10.20 (8)</td>
</tr>
<tr>
<td></td>
<td>Danazol</td>
<td>7.10 ± 1.17 (4)</td>
<td>11.70 ± 2.57 (4)</td>
<td>4.93 ± 1.50 (4)</td>
<td>0.08 ± 0.02* (4)</td>
<td>6.48 ± 10.22 (4)</td>
</tr>
<tr>
<td></td>
<td>MPA</td>
<td>8.46 ± 1.56 (7)</td>
<td>6.59 ± 1.07* (7)</td>
<td>7.41 ± 1.45 (7)</td>
<td>0.16 ± 0.06 (7)</td>
<td>0.88 ± 0.26** (6)</td>
</tr>
</tbody>
</table>

Asterisks indicate significant differences between control and treatment values on the same day of the cycle (* P < 0.05; ** P < 0.01).
which large doses of danazol were given alone or in combination with progesterone to rabbit for 5 days (Kokko, submitted for publication). Danazol alone was able to induce a dose-dependent synthesis of uteroglobin, a progesterone-specific rabbit uterine protein, and brought about changes in cytosol and nuclear PR concentrations typical of progesterone action in the rabbit uterus (Isomaa et al. 1979). When given concomitantly with progesterone, the action of danazol was additive rather than inhibitory to the progesterone-elicted changes in uteroglobin secretion and cytosol and nuclear PR levels. 

Danazol caused an increase in serum LH concentration on day 6 of the treatment cycle. The significant decrease in serum oestradiol in samples taken close to the end of the cycle corroborates studies showing danazol to inhibit various steps in steroidogenesis (Barbieri et al. 1977a,b; Tsang et al. 1979). In concert with previous studies (Chimbira et al. 1980; Asch et al. 1980), danazol also tended to interfere with ovulation and/or progesterone biosynthesis in the corpus luteum leading to low serum progesterone on day 24 of the cycle. The possibility therefore exists that the changes in endometrial receptor concentrations and 17ß-HSD activity are mediated via a decrease in the circulating sex steroids, rather than being direct progesterational effects of danazol or MPA. This alternative seems unlikely, since the present changes in cytosol ER and PR levels during MPA administration were identical with those seen during the same treatment of post-menopausal patients with endometrial hyperplasia or carcinoma (Jänne et al. 1979).

Collectively, on the basis of our results it is very difficult to assign an antiprogestational activity for danazol. Rather, when danazol is given in doses used for the treatment of endometriosis (Dmowski 1979; Jenkin 1979), it exhibits actions that are very similar to those seen during a long-term treatment with high doses of an active progestin such as MPA.

Acknowledgments

We are grateful for the skilful technical assistance of Mrs. Sirkka Ekdahl. Danazol (Danocrine®) was kindly donated by Sterling-Winthrop. This investigation was supported by a grant from The National Research Council for Medical Sciences, Finland.

References


Jänne O, Kauppila A, Kontula K, Syrjälä P & Vihko R (1979): Female sex steroid receptors in normal, hyperplastic and carcinomatous endometrium. The relation-
ship to serum steroid hormones and gonadotrophins and changes during medroxyprogesterone acetate administration. Int J Cancer 24: 545–554.


Received on April 29th, 1981.