

Danazol concentrations in ovary, uterus, and serum and their effect on the hypothalamic-pituitary-ovarian axis during vaginal administration of a danazol suppository*

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Objective: To determine danazol concentrations in the ovary, uterus, and serum during daily vaginal administration of a danazol suppository and to examine its effect on the hypothalamic-pituitary-ovarian axis.

Design: Sampling of tissues after vaginal or oral administration of danazol and sampling of blood during control and danazol-administration menstrual cycles.

Setting: Outpatient volunteers and inpatients at a public hospital.

Participants: Thirty patients who were to undergo hysterectomy and oophorectomy because of uterine leiomyoma and eight regularly menstruating volunteers.

Interventions: Danazol was administered as a vaginal suppository (100 mg) or orally (400 mg).

Main Outcome Measure: Danazol concentrations in the ovary, uterus, and serum, and serum E₂ and P levels.

Results: Danazol concentrations in the ovary and uterus after daily vaginal administration of a suppository containing 100 mg danazol were comparable to those after daily oral administration of 400 mg danazol, but the serum danazol concentration was much lower. Menstrual cycle patterns of serum E₂ and P levels were normal during daily vaginal administration of a danazol suppository.

Conclusion: Daily administration of a suppository containing 100 mg danazol produces high ovarian and uterine concentrations but low serum concentrations, and no effect was detected on the hypothalamic-pituitary-ovarian axis. *Fertil Steril* 1995;63:1184-9

Key Words: Danazol, suppository, vaginal administration, danazol concentration, serum estradiol, serum progesterone, ovary, uterus

Danazol, a synthetic derivative of 17 α -ethinyltestosterone, has been used widely in the treatment of endometriosis (1, 2). Oral administration of 400 to 600 mg/d results in high serum concentrations of danazol, which may produce androgenic side effects, such as acne, hirsutism, and weight gain (3, 4). Da-

nazol has androgenic activity (5) and increases serum free T by decreasing serum sex hormone-binding globulin (SHBG) (6, 7). Furthermore, because orally administered danazol is absorbed from the gastrointestinal tract and enters the liver via the portal vein, liver dysfunction may result (3). The severity of these side effects sometimes compels patients to discontinue treatment with oral danazol.

Recently, Igarashi (8) reported that intravaginal administration of a danazol ring containing 2 to 3.5 g of danazol was effective in treatment of endometriosis and that intravaginal administration resulted in much lower serum levels of danazol than oral administration of 400 mg of danazol once per day. These results suggest that vaginal administration can serve as an alternative route for danazol therapy

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that might produce fewer and less severe side effects. Therefore, we prepared a suppository containing 100 mg of danazol to treat patients with endometriosis by intravaginal administration. In the present study, we measured danazol concentrations in the ovary, uterus, and serum after daily vaginal administration of a danazol suppository and investigated its effects on the hypothalamic-pituitary-ovarian axis.

MATERIALS AND METHODS

Subjects

Subjects in this study were women with regular menstrual cycles who were between 23 and 43 years of age. Thirty patients (47.5 ± 2.3 years; mean \pm SEM) who were to undergo hysterectomy and unilateral oophorectomy because of uterine leiomyoma underwent assay of danazol concentrations in the ovary, uterus, and serum. Two volunteers (31 and 33 years) and six volunteers (31.2 ± 5.1 years) underwent assay of serum E₂ and P levels throughout menstrual cycles and at the midluteal phase, respectively. Informed consent was obtained from all patients.

Preparation of a Danazol Suppository

One hundred milligrams of danazol was suspended in 1 mL of vosco-H 15 (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) at 60°C. The suspension was transferred into a cylindrical container, where it turned solid at room temperature.

Measurement of Danazol Concentrations in Ovary, Uterus, and Serum

Thirty patients who were to undergo hysterectomy and unilateral oophorectomy were divided into three groups. Patients of group I received a danazol suppository in the posterior vaginal fornix 4, 12, or 36 hours before hysterectomy and oophorectomy. Patients of group II were instructed to insert a danazol suppository into the posterior vaginal fornix by themselves every day for 30 days until surgery. Patients in group III took a capsule containing 200 mg of danazol twice per day (400 mg/d) for 30 days until surgery. The uterus and ovary were removed at surgery 12 hours after the last administration of danazol in groups II and III. Blood was drawn at hysterectomy and oophorectomy in each group, and serum was separated by centrifugation. Serum, endometrium and myometrium of the uterine cervix and corpus, and the ovary were stored at -20°C until the assay for danazol. Endometrium, myometrium, and ovaries were homogenized with four volumes of saline in a Polytron PT10 homogenizer (Brinkman In-

struments, Westbury, NY). Myometrium and ovaries were minced with scissors before homogenization. Danazol in the tissue homogenate and in serum was extracted with a solution of n-hexane-chloroform (7:3, vol/vol), and then applied to a Sep-Pack Silica cartridge absorption column (Millipore Co., Bedford, MA). The absorbed danazol was eluted with chloroform. After evaporation of chloroform, danazol was resolved in a small amount of the n-hexane-chloroform solution and measured by high-pressure liquid chromatography with a Lichrosorb Si 605 column (250 \times 4.6 cm; Merck & Co., Inc. Rahway, NJ).

Serum E₂ and P Levels During Daily Vaginal Administration of a Danazol Suppository

Eight volunteers with regular menstrual cycles received a danazol suppository in the posterior vaginal fornix once per day, from the 4th day of each menstrual cycle to the beginning of menstruation, for two to six successive menstrual cycles. The menstrual cycle immediately before the menstrual cycle in which danazol was administered was regarded as a control cycle. Two volunteers had blood drawn to measure serum E₂ and P levels every 2nd day during the control menstrual cycle and the second menstrual cycle of danazol administration; BBT was recorded during both cycles. Six volunteers had blood drawn to measure serum E₂ and P levels at the midluteal phase (7 to 9 days after LH surge) of both the control menstrual cycle and the second menstrual cycle of danazol administration. The LH surge was determined by measurement of urine LH levels and by observation of follicular development by transvaginal ultrasonography.

Endometrial Histology During Daily Vaginal Administration of a Danazol Suppository

Endometrial biopsy was performed on the 5th day after ovulation of the second menstrual cycle of danazol administration in each of the volunteers. Time of ovulation was confirmed by LH surge and disappearance of Graffian follicles on ultrasonography.

Assay of Serum E₂ and P levels

Serum E₂ and P levels were determined by RIA kits (Diagnostic Products Co., Los Angeles, CA). The intra-assay and interassay coefficients of variation were 6.0% and 6.8% for E₂ and 5.8% and 6.5% for P.

Detection of Urine LH

Urine LH concentration was measured with an L-CHECK enzymeimmunoassay kit (Nipro Co., Osaka, Japan). An LH concentration > 40 mIU/mL (conversion factor to SI unit, 1.00) was considered positive.

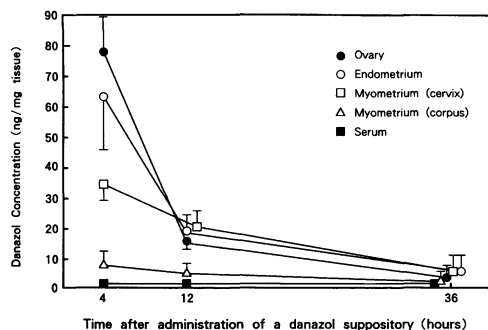


Figure 1 Time course of danazol concentrations in the ovary, endometrium and myometrium of the uterine cervix and corpus, and serum after single vaginal administration of a danazol suppository. Each point represents a mean \pm SEM of three to five samples.

Statistics

Values are presented as means \pm SEM. Statistical significance ($P < 0.05$) was evaluated by Student's t -test and the paired t -test.

RESULTS

Danazol Concentrations in Ovary, Uterus, and Serum After a Single Vaginal Administration of Danazol Suppository

Danazol concentrations in the ovary, uterus, and serum after a single vaginal administration of danazol suppository are shown in Figure 1. Danazol administered via the vagina is distributed rapidly to the ovaries and uterus, and danazol concentrations in the ovary and uterus reached high levels 4 hours after administration, in order of decreasing concentration, in the ovary, endometrium, myometrium of the uterine cervix, and myometrium of the uterine corpus. Danazol concentrations in these tissues 12 hours after administration decreased to one third to one fourth the levels after 4 hours, and those 36 hours after the danazol administration were <5 ng/mg tissue. In contrast, serum danazol concentrations in all patients were always <1 ng/mL.

Danazol Concentrations in the Ovary, Uterus, and Serum After Daily Vaginal Administration of a Danazol Suppository

Figure 2 shows danazol concentrations in the ovary, uterus, and serum 12 hours after the last administration of danazol in patients who received either a single vaginal suppository containing 100 mg of danazol, a daily vaginal danazol suppository for 30 days, or daily oral administration of 400 mg of danazol for 30 days. Danazol concentrations in the ovary and uterus after daily vaginal administration of 100 mg of danazol were two to three times higher

than those after a single administration and were comparable to those after daily oral administration of a fourfold higher dose (400 mg). However, the serum danazol level after daily vaginal administration of danazol was $<\frac{1}{20}$ of that after daily oral administration.

Serum E_2 and P Levels During Daily Vaginal Administration of a Danazol Suppository

Eight volunteers with regular menstrual cycles received a vaginal danazol suppository once per day for two to six successive menstrual cycles. Figure 3 shows BBT and serum levels of E_2 and P during the control menstrual cycle and the second menstrual cycle of danazol administration of one volunteer. During the control menstrual cycle, BBT showed a biphasic pattern, and LH surge occurred, as shown by urinary LH levels. Serum E_2 and P levels also showed normal patterns (Fig. 3A). During the second menstrual cycle of danazol administration, BBT and serum levels of E_2 and P showed patterns similar to those during the control menstrual cycle, and LH surge was confirmed (Fig. 3B). Follicular development in the ovaries and ovulation were confirmed

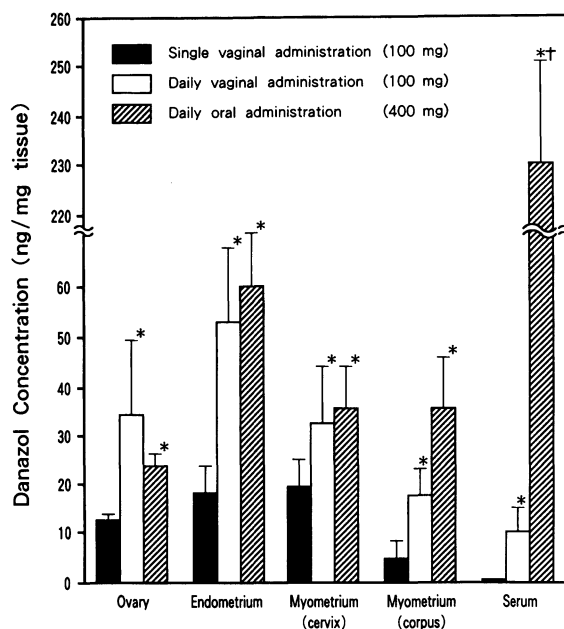
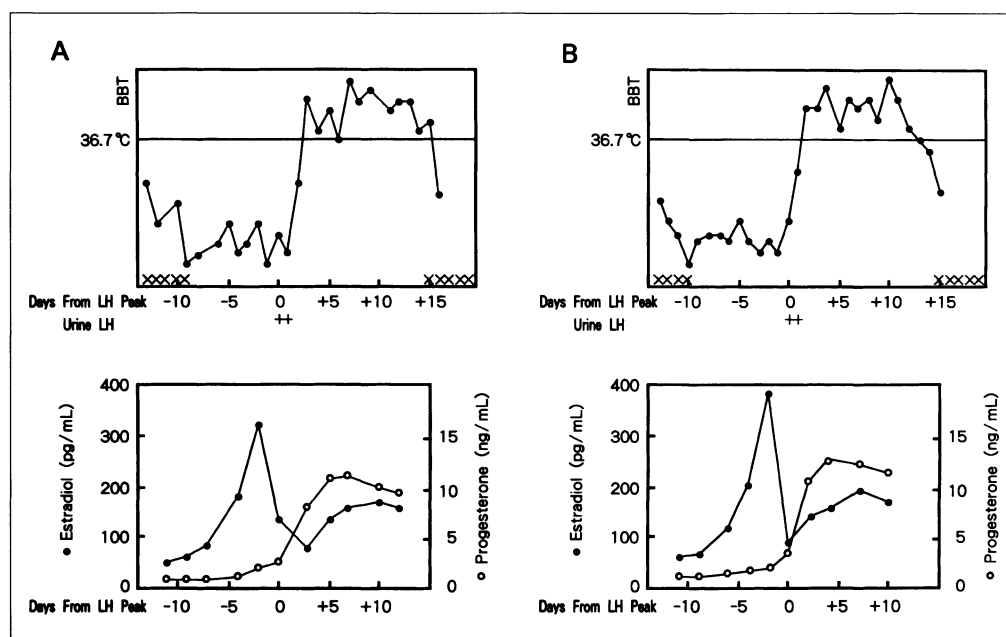


Figure 2 Danazol concentrations in the ovary, endometrium and myometrium of the uterine cervix and corpus, and serum after single vaginal administration of 100 mg danazol, daily vaginal administration of 100 mg danazol for 30 days, and daily oral administration of 400 mg danazol for 30 days. Danazol concentrations were measured 12 hours after the last administration. The height of a bar represents a mean \pm SEM of three to five samples. * $P < 0.05$, significant difference from the values after single vaginal administration of danazol by Student's t -test. † $P < 0.05$, significant difference from the values after daily vaginal administration of danazol by Student's t -test.

Figure 3 The BBT and serum E_2 and P levels during the control menstrual cycle (A) and the second menstrual cycle (B) of daily vaginal administration of danazol (conversion factors to SI unit for E_2 and P are 3.617 and 3.180, respectively).



by transvaginal ultrasonography during both the control menstrual cycle and the second menstrual cycle of danazol administration. Results in a second volunteer were similar (data not shown).

Serum E_2 and P levels of the other six volunteers were measured at the midluteal phase in the control menstrual cycle and the second menstrual cycle of danazol administration. Serum E_2 levels of these volunteers in the control cycle versus those in the second cycle of danazol administration were 158 versus 108, 119 versus 150, 121 versus 102, 111 versus 120, and 95 versus 124 pg/mL (conversion factor to SI unit, 3.617), respectively; the means \pm SEM of the former and the latter were 120.8 ± 10.4 and 120.8 ± 8.3 , respectively. Their serum P levels in the control cycle versus those in the second cycle of danazol administration were 11.5 versus 10.5, 11.0 versus 11.2, 10.8 versus 9.3, 8.2 versus 7.9, 8.0 versus 12.8, and 6.0 versus 8.1 ng/mL (conversion factor to SI unit, 3.180), respectively; the means \pm SEM of the former and the latter were 9.3 ± 0.9 and 10.0 ± 0.8 , respectively. There were no significant differences in serum E_2 and P levels between the cycles when examined by the paired *t*-test. Of the eight volunteers, six received a danazol suppository for three to six successive menstrual cycles (total, 24 cycles). The BBT of each menstrual cycle showed the normal biphasic pattern, and no menstrual disorders were found. Furthermore, no acne, hirsutism, or weight gain was found in five volunteers. Only one volunteer complained of very mild acne. Serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels of these six volunteers

after the last administration of danazol suppository were 9, 13, 15, 7, 13, and 10 IU/L, and 6, 10, 8, 6, 11, and 8 IU/L, respectively, and were within normal ranges (normal ranges of GOT and GPT were 7 to 34 and 4 to 32 IU/L, respectively).

Endometrial Histology During Vaginal Administration of a Danazol Suppository

To investigate the direct effect of vaginally administered danazol on endometrium, endometrial biopsy was performed in the eight volunteers on the 5th day after the ovulation of the second menstrual cycle of vaginal danazol administration. A representative endometrial histologic pattern is shown in Figure 4. The endometrium was abundant in tortuous glands, and the accumulation of secretion was found often in their lumen. In some glands the nuclei of epithelial cells displaced toward the free surface, forming subnuclear vacuoles, but in the rest of glands their nuclei were found in the basal cytoplasm. The stroma of the endometrium was partly edematous. These histologic developments correspond to the early secretory phase.

DISCUSSION

Orally administered danazol is absorbed from the gastrointestinal tract, enters the systemic circulation via the portal vein, and then is distributed to various tissues, including the ovaries and uterus. Therefore, oral administration of danazol results in high serum concentrations, as shown in the present study. In contrast, a single vaginal administration

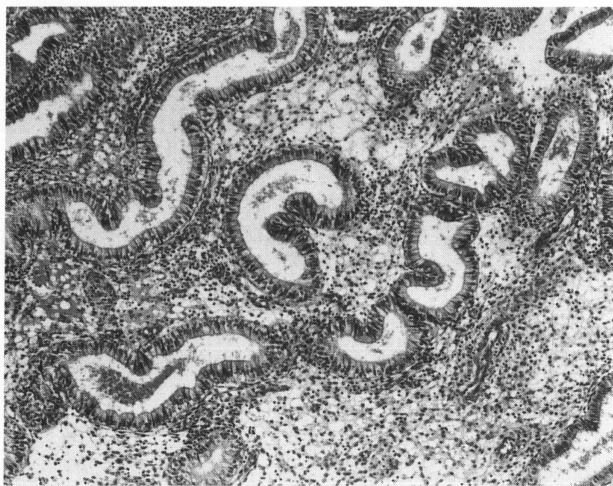


Figure 4 Endometrium on the 5th day after the ovulation during the second menstrual cycle of daily vaginal administration of danazol. Hematoxylin and eosin, Magnification, $\times 71$.

of danazol produced high danazol concentrations in the ovary and uterus while keeping the serum danazol concentration at much lower levels. Furthermore, danazol concentrations in the ovary and uterus after daily vaginal administration (100 mg) were comparable to those after daily oral administration at a fourfold higher dose (400 mg), whereas the serum danazol concentration after daily vaginal administration was $< \frac{1}{20}$ of that after daily oral administration. Therefore, it is likely that vaginally and orally administered danazol reach the ovary and uterus through different routes. A venous plexus or the lymphatic system is the probable route from the vagina to these organs.

Danazol has antigonadotropic activities, and daily oral administration of 400 mg of danazol inhibits both ovulation and LH surge (9–11). However, neither was inhibited by daily vaginal administration. This result is attributable to the lower serum concentration of danazol produced by vaginal administration.

Daily vaginal administration of 100 mg danazol had no effect on either serum levels of E_2 and P or menstrual cycles, whereas the danazol concentration in the ovary was comparable with that from daily oral administration of 400 mg danazol. These results suggest that danazol at concentrations produced by daily oral administration of 400 mg danazol does not inhibit estrogen production by the ovaries. Therefore, the decreased serum levels of E_2 after daily administration of 400 mg danazol reported by several investigators (9, 12, 13) apparently were due to the action of danazol at high serum concentrations on the hypothalamic-pituitary axis (13, 14). On the other hand, there are many reports that danazol in-

hibited steroidogenesis in the ovary in vitro (15–17). In these studies, danazol was used at concentrations greater than $1 \mu\text{M}$. However, according to the present and previous studies (18), danazol concentrations in the ovary after oral administration of 400 mg danazol are $< 0.3 \mu\text{M}$. Therefore, the extrapolation of in vivo effects of danazol from in vitro studies requires great care.

Endometrium during daily vaginal administration of 100 mg danazol was not atrophic. Because daily vaginal administration of danazol did not affect serum levels of E_2 and P, this finding suggests that danazol at a concentration reached in the endometrium during daily vaginal administration (100 mg) does not inhibit the actions of estrogen and P there.

Danazol at high serum concentrations during daily oral administration sometimes produces side effects, such as acne, hirsutism, and weight gain (3, 4). In contrast, daily vaginal administration of danazol resulted in much lower serum concentrations, and only mild acne was found in one of six volunteers. Moreover, serum GOT and GPT levels of volunteers were within normal ranges. Thus, daily vaginal administration of danazol would be accompanied by fewer and less severe side effects than daily oral administration. In the present study, the effectiveness of daily vaginal administration of danazol in the treatment of endometriosis was not examined. However, Igarashi (8) reported that a single vaginal administration of a danazol ring containing 2 to 3.5 g of danazol reduced dysmenorrhea and the extent of pelvic endometriosis in each of 35 women treated. Therefore, vaginal administration could be an alternative therapeutic route with fewer and less severe side effects. Furthermore, if the effectiveness of vaginal administration of danazol for treatment of endometriosis is confirmed, it would indicate that danazol has a direct effect on endometriotic tissue because vaginal administration of danazol does not affect the hypothalamic-pituitary-ovarian axis, although danazol may not have a direct effect on the endometrium as suggested in this study. The effectiveness of oral administration of danazol for treatment of endometriosis may be ascribed to a direct effect of danazol on endometriotic tissue and to its effect on the hypothalamic-pituitary axis.

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