Endocrine Journal 1993, **40** (6), 659–664

Adrenal Steroids in Serum during Danazol Therapy, Taking into Account Cross-Reactions between Danazol Metabolites and Serum Androgens

KOUICHI MURAKAMI, TOSHINOBU NAKAGAWA,
GEN YAMASHIRO, KATSUMI ARAKI, AND KAZUTOMO AKASOFU

Department of Obstetrics and Gynecology, Kanazawa University School of Medicine, Kanazawa 920, Japan

Abstract. To investigate the changes in the serum androgen concentrations and the Free Androgen Index (FAI) in women during danazol therapy, we measured the serum concentrations of adrenal steroids and danazol metabolites, and then examined the effects of danazol metabolites on assays for serum androgens. Thirteen women who had endometriosis were treated with danazol (300 or 400 mg/day) for 8 to 16 weeks. Blood samples were taken before, during, and after the medication. During the danazol therapy, serum testosterone (T), cortisol (F), and sex-hormone binding globulin (SHBG) significantly decreased ($P<0.05$); but serum dehydroepiandrosterone-sulfate (DHEAS) and FAI increased ($P<0.05$). The serum concentrations of danazol metabolites were: danazol, 209.0±28.3 (ng/mL, mean ± SEM); Δ^1-2-hydroxymethyl ethisterone, 114.4±8.4; and 2-hydroxymethyl ethisterone, 660.0±54.2. There was considerable cross-reaction between danazol metabolites and androgens [T, androstenedione (A), and dehydroepiandrosterone (DHEA)] in the direct assays. As for the ratios of adrenal steroids in serum, the DHEAS/F, DHEAS/DHEA, and 11-deoxycortisol (S)/F ratios increased ($P<0.05$). We conclude that the increase in FAI and DHEAS represents increased native androgenic activity with danazol, and the changes in adrenal steroid ratios in serum indicate the inhibition of 11β-hydroxylase and sulfatase activities during danazol therapy.

Key words: Danazol, Adrenal androgens, Endometriosis. (*Endocrine Journal* **40**: 659–664, 1993)

DANAZOL, an isoxazole derivative of the synthetic steroid 17-ethinyltestosterone (ethisterone), is being used clinically in the management of endometriosis, chronic cystic mastitis, and endometrial adenomatous hyperplasia. It has been reported that the mechanisms of action of danazol involve antigonadotropic effects, inhibition of steroidogenesis, facilitation of steroid metabolism and direct androgenic effects, but the detailed pharmacology of danazol is still unclear [1].

Native androgens have many biological effects throughout a woman's life. To investigate the changes in serum androgen levels and the Free Androgen Index (FAI) in women during danazol therapy, it is very important to determine the serum concentrations of androgens (both ovarian and adrenal) and danazol metabolites during danazol therapy. There are numerous studies regarding serum androgen levels during danazol therapy [1–8], but only a few of these consider serum levels of adrenal androgens [2, 4, 8]. Adrenal androgens in serum, predominantly composed of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS) in humans, increase with adrenarche, reach a maximum in the second and third decades of life, and decline with advancing years. Because of the unchanged serum cortisol (F) levels throughout a lifetime, the changes in DHEAS/F and DHEA/F ratios have been used as indices of aging.

To correctly measure concentrations of serum

Received: June 25, 1993
Accepted: September 17, 1993
Correspondence to: Dr. Kouichi MURAKAMI, Department of Obstetrics and Gynecology, Kanazawa University School of Medicine, 13–1 Takaramachi, Kanazawa 920, Japan.

androgens by radioimmunoassay (RIA), we must reduce the cross-reacting materials as much as possible. Considerable amounts of cross-reacting steroids including danazol metabolites have been detected in RIA for serum androgens (especially for testosterone) [2], but they have not been investigated fully.

In this study, we investigated the serum concentrations of androgens and other adrenal steroids during danazol therapy as well as the influence of danazol on these assays; in addition, we considered the changes in adrenal steroid ratios in serum during danazol therapy.

Materials and Methods

Patients

Thirteen women who had endometriosis were selected for this study and informed consent was obtained from each. The patients ranged from 26 to 48 years in age, averaging 35.5 years. The menstrual cycles of 10 patients were regular before danazol therapy, while the others were irregular. The patients were treated with danazol (300–400 mg/day) for 8 to 16 weeks. Blood samples were collected before treatment (in the early follicular phase), during treatment (in the 2nd, 4th, 8th, and 12th weeks), and 1 month after the end of treatment. The blood samples were withdrown between 0900 h and 1000 h with the patient in the supine position at bed rest (2 to 3 h after the last danazol administration); the serum was stored at −80°C until the assay.

Hormone assays

Serum DHEAS were measured by solid-phase RIA (Coat-A-Count kit, Diagnostic Products Corporation, Los Angeles, CA). Serum F was measured by RIA (SPAC Cortisol Kit II, Daiichi Radioisotope Labs. Tokyo, Japan). Serum DHEA and androstenedione (A) were extracted with ethyl ether, and separated by Sephadex LH-20 (Pharmacia, Uppsala, Sweden) column chromatography (hexane: benzene: methanol = 8:1:1); then they were determined by RIA with anti-DHEA antibody (kindly supplied by Dr. Sekihara, 3rd Department of Internal Medicine, Tokyo University, Tokyo), and anti-A antibody (Teikoku Zouki Co., Ltd. Tokyo, Japan). Serum 11-deoxycortisol (S) was also determined after dichloromethane extraction and Sephadex LH-20 separation (benzene: methanol = 98:2) by RIA with anti-S antibody (Teikoku Zouki Co., Ltd.). Serum testosterone (T) was measured with a commercial kit (CIS kit, CIS Diagnostic Co., Ltd. Sakura, Japan) with or without dichloromethane extraction followed by high-performance liquid chromatography (HPLC, LC-6A; Shimazu Co. Kyoto, Japan and Zorbax CN column, 4.6 mm × 25 cm; Shimazu Co.; methanol: acetonitrile: H_2O = 3:2:7). Serum danazol and danazol metabolites (ethisterone, $\triangle^1$-2-hydroxymethyl ethisterone, 2-hydroxymethyl ethisterone) were extracted with dichloromethane and were measured by HPLC. Sex hormone binding globulin (SHBG) was assayed with an immunoradiometric assay kit (Farmos Diagnostica Co., Ltd. Oulunsalo, Finland), and the FAI was calculated as the total T concentration (nmol/L) × 100, divided by the SHBG concentration (nmol/L). The intra-assay and inter-assay coefficients of variation were <10% and 15%, respectively, for all of these assays.

The cross-reactions with danazol metabolites were examined on each assay.

The ratios of respective steroids in serum during danazol therapy were calculated from the data obtained from the above assays.

Statistical methods

Statistical analysis was performed by Student's *t*-test. Data are expressed as mean ± SEM.

Results

The serum DHEAS levels were significantly higher during danazol therapy than in the pretreatment period ($P<0.05$). Serum DHEA decreased slightly, and the serum A levels were unchanged during treatment. Serum T levels during danazol therapy measured by RIA without extraction and separation were significantly higher ($P<0.05$) than levels in the pretreatment period. When measured after extraction and HPLC separation, however, these levels were significantly lower than in the pretreatment period ($P<0.05$). The SHBG levels were 83.9 ± 17.7 nmol/L before

treatment, and decreased markedly to 6.5 ± 0.5 nmol/L during the treatment ($P<0.05$), but the FAI increased significantly during danazol therapy ($P<0.05$). Serum concentrations (ng/mL) of danazol metabolites were: danazol, 209.0 ± 28.3; Δ^1-2-hydroxymethyl ethisterone, 114.4 ± 8.4; and 2-hydroxymethyl ethisterone, 660.0 ± 54.2. No serum ethisterone was detected in this assay (Table 1).

As for the cross-reactions between T and danazol metabolites in the T kit, the percentage of cross-reactions was 0.2% for danazol, 0.08% for Δ^1-2-hydroxymethyl ethisterone, 0.2% for 2-hydroxymethyl ethisterone and 1.1% for ethisterone. DHEA and A also showed cross-reactions, which were, however, lower than those for T. DHEA and A were separated from danazol metabolites by Sephadex LH-20 column chromatography (data not shown). In the DHEAS kit, the percentage of cross-reactions was <0.001% (Table 2).

Serum F decreased from 14.4 ± 1.6 (μg/dL) to 7.9 ± 0.8 in the 2nd week and remained low during the rest of the treatment period ($P<0.05$). Serum S was unchanged during danazol treatment. The DHEAS/F, DHEAS/DHEA and S/F ratios increased during the treatment period ($P<0.05$). (Fig. 1).

Discussion

Danazol has been used clinically for the management of endometriosis for more than 15 years. The clinical effects of danazol have been attributed to its antigonadotropic and androgenic properties, but the relationship between serum levels of androgens and danazol metabolites during danazol therapy has not been investigated fully.

Native androgens have many biological effects on women. Serum androgens in premenopausal

Table 1. Concentrations of androgens and danazol metabolites in serum during danazol therapy[a)]

	Before	During[b)]	After
DHEAS (μg/dL)	105.0±16.3	211.9±22.6[c)]	182.9±40.9[c)]
DHEA (ng/mL)	3.31±0.58	2.64±0.23	3.95±0.69
A (ng/mL)	0.79±0.14	0.78±0.1	0.86±0.21
T (ng/mL)			
direct assay	0.34±0.05	6.14±0.39[c)]	0.40±0.06
with HPLC	0.32±0.06	0.22±0.04[c)]	0.38±0.05
SHBG (nmol/L)	83.9±17.7	6.5±0.5[c)]	37.9±3.7
FAI	2.0±0.5	14.8±0.2[c)]	4.4±0.9
Danazol (ng/mL)		209.0±28.3	
Δ^1-2-hydroxymethyl Ethisterone (ng/mL)		114.4±8.4	
2-hydroxymethyl Ethisterone (ng/mL)		660.0±54.2	
Ethisterone (ng/mL)		ND[d)]	

DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; A, androstenedione; T, serum testosterone; SHBG, sex hormone binding globulin; FAI, free androgen index. a) Values are the means±SEM. b) Data are the totals for the 2nd, 4th, 8th, and 12th weeks. c) $P<0.05$ with reference to pretreatment values. d) ND, not detectable.

Table 2. Percentage cross-reactions between danazol metabolites and serum androgens

	Danazol	Δ^1-2-hydroxymethyl Ethisterone	2-hydroxymethyl Ethisterone	Ethisterone
T	0.2	0.08	0.2	1.1
A	0.008	0.01	0.008	0.005
DHEA	<0.001	<0.001	0.005	0.01
DHEAS	<0.001	<0.001	<0.001	<0.001

T, serum testosterone; A, androstenedione; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate.

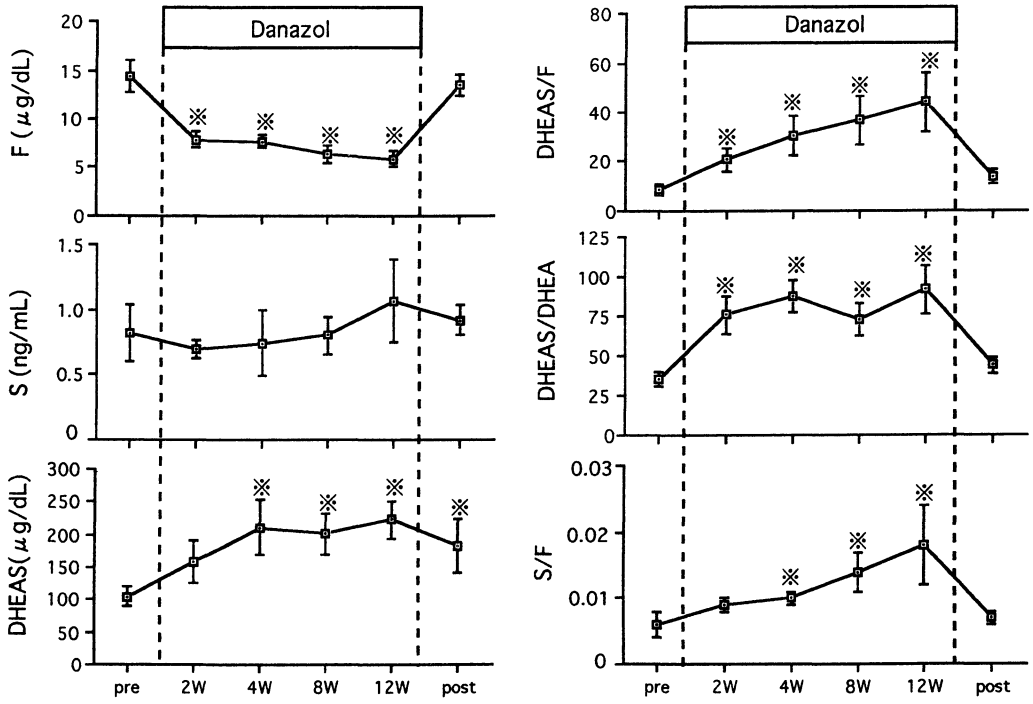

Fig. 1. Serum concentrations of F, S, and DHEAS (left panel) and the DHEAS/F, DHEAS/DHEA and S/F ratios in serum (right panel) during danazol therapy. Each point represents the mean ± SEM. ※, $P<0.05$ with reference to pretreatment values.

women derive from the ovarium and the adrenal gland, and especially adrenal androgens are secreted abundantly in young women, so it is necessary to investigate the changes in the serum concentrations of both types of androgens in women. Native androgens may inhibit the growth of endometriotic tissues; therefore, to examine the androgenic effects of danazol at tissue level, it is also important to investigate the serum concentrations of native androgens, especially those of free androgens. Although the precise functions of adrenal androgens are uncertain, there are some studies of the relation between adrenal androgens and Alzheimer's disease [9–11], osteoporosis [12], cardiovascular disease [13], and human immunodeficiency virus infection [14]. On the other hand, the DHEAS/F or DHEA/F ratio is particularly thought of as a discriminator of degradative changes in humans; it is meaningful for studying the process of aging to investigate the changes in the DHEAS/F or DHEA/F ratio due to diseases and medications.

There have been many studies giving contradictory findings in relation to the serum T concentration during danazol therapy. Some investigators report an increase in T [2, 3, 6], but others report that T decreased during treatment [1, 4, 5, 7, 8, 15]. The present results obtained from the assays performed after extraction and HPLC separation show that the serum T concentration is low during danazol therapy; but the direct assay resulted in values higher than the pretreatment one. This discrepancy in the serum T concentration could be attributed to cross-reactions with danazol metabolites in the direct assay. In our findings, serum danazol metabolites are responsible for about 30–40% of the cross-reactions in T kits. Danazol metabolites other than danazol, 2-hydroxymethyl ethisterone and Δ^1-2-hydroxymethyl ethisterone may be responsible for the remaining 60–70% of cross-reactions. Further investigation is needed to clarify this point. The main cause of a decrease in serum T during danazol therapy may be the decrease in ovarian T production, but the effects of the decreased serum adrenal androgens must also be considered. Another cause of the decrease in serum T may be a decrease in SHBG during danazol therapy. Danazol profoundly decreases the SHBG concentration and also competes with T in binding to SHBG [15]. Consequently, the metabolism of T may be increased. These events may lead to an increase in free T in serum. This

hypothesis is supported by the increase in FAI during danazol therapy in the present study.

Serum DHEA and A decreased slightly but DHEAS increased during danazol therapy in the present study. Stillman *et al.* [7] report increasing serum DHEA and A during danazol therapy, but Sherins *et al.* [4] and Carlström *et al.* [8] report decreased serum DHEA and A. The decrease in serum DHEA and A may reflect the inhibitory effects of danazol on steroidogenesis. As for the increase in serum DHEAS, Carlström *et al.* [16] report that hepatic sulfatase was inhibited during danazol therapy. A decrease in adrenal sulfatase activity has also been proposed, but this point was not clarified in our study. The increase in the DHEAS/DHEA ratio during danazol therapy represents a decrease in conversion from DHEAS to DHEA caused by sulfatase inhibition, but it is necessary to investigate the changes of sulfokinase and 3β-hydroxysteroid dehydrogenase/$\triangle^{4,5}$-isomerase activities.

Decreased serum F and an increase in the S/F ratio were seen in the present study. The serum concentration of adrenocorticotropin (ACTH) was unchanged (data not shown), and serum S also did not vary in the present study. These data suggest that 11β-hydroxylase is inhibited by danazol in the adrenal glands. On the other hand, the changes in the metabolic clearance rate of F must also be considered factor contributing to the decrease in serum F. The decrease in corticosteroid binding globulin (CBG) during danazol therapy [17] may also affect the serum F concentration.

The increased DHEAS/F ratio during danazol therapy is thought to be caused by the inhibition of sulfatase and 11β-hydroxylase by danazol. The decrease in the DHEAS/F or DHEA/F ratio has been thought to reflect aging. Thus danazol may reduce the effect of aging on endocrinological function.

Acknowledgments

We thank Miss Masako Deguchi for her technical assistance and Tokyo Tanabe Co., Ltd. for kindly supplying danazol and danazol metabolites.

References

1. Barbieri RL, Ryan KJ (1981) Danazol: Endocrine pharmacology and therapeutic applications. *Am J Obstet Gynecol* 141: 453–463.
2. Floyd WS (1980) Danazol: Endocrine and Endometrial Effects. *Int J Fertil* 25 (1): 75–80.
3. Hirschowitz JS, Soler NG, Wortsman J (1979) Sex steroid levels during treatment of endometriosis. *Obstet Gynecol* 54: 448–450.
4. Sherins RJ, Gandy HM, Thorslund TW, Paulsen CA (1970) Pituitary and testicular function studies. I. Experience with a new gonadal inhibitor, 17α-pregn-4-en-20-yno-(2, 3-d) isoxazol-17-ol (Danazol). *J Clin Endocr* 32: 522–531.
5. Steingold KA, Lu JKH, Judd HL, Meldrum DR (1986) Danazol inhibits steroidogenesis by the human ovary *in vivo*. *Fertil Steril* 45: 649–654.
6. Nakamura K, Kondou I, Hayashi T, Kimura T, Oosawa M, Narita O (1992) Circulating androgen dynamics and skin lipids and acne during danazol therapy. *Jpn J Fertil Steril* 37: 56–62 (In Japanese).
7. Stillman RJ, Fench MD, Barbieri RL, Tulchinsky D (1980) Inhibition of adrenal steroidogenesis by danazol *in vivo*. *Fertil Steril* 33: 401–406.
8. Carlström K, Döbel A, Gershagen S, Rannevik G (1984) Peripheral levels of dehydroepiandrosterone sulfate, dehydroepiandrosterone, androstenedione and testosterone following different doses of danazol. *Acta Obstet Gynecol Scand Suppl* 123: 125–129.
9. Lacroix G, Fiet J, Benais JP, Gueux B, Bonete R, Villette JM, Gourmel B, Dreux C (1987) Simultaneous radioimmunoassay of progesterone, androst-4-enedione, pregnenolone, dehydroepiandrosterone and 17-hydroxyprogesterone in specific regions of human brain. *J Steroid Biochem* 28: 317–325.
10. Sunderland T, Merril CR, Harrington MG, Lawlor BA, Molchan SE, Martinez R, Murphy DL (1989) Reduced plasma dehydroepiandrosterone concentrations in Alzheimer's disease. *Lancet* 570.
11. Svec F, Lopez-s A (1989) Antiglucocorticoid actions of dehydroepiandrosterone and low concentrations in Alzheimer's disease. *Lancet* 1335–1336.
12. Wild RA, Buchahan JR, Myers C, Demers LM (1987) Declining adrenal androgens: An association with bone loss in aging women. *Proc Soc Exper Bio Med* 186: 355–360.
13. Connor EB, Khaw KT, Yen SSC (1986) A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. *N Engl J Med* 315: 1519–1524.
14. Villette JM, Bourin P, Doinel C, Mansour I, Fiet J,

Boudou P, Dreux C, Roue R, Debord M, Levi F (1990) Circadian variations in plasma levels of hypophyseal, adrenocortical and testicular hormones in men infected with human immunodeficiency virus. *J Clin Endocrinol Metab* 70: 572–577.

15. Dowsett M, Forbes KL, Rose GL, Mudge JE, Jeffcoate SL (1986) A comparison of the effects of danazol and gestrinone on testosterone binding to sex hormone binding globulin *in vitro* and *in vivo*. *Clin Endocrinology* 24: 555–563.
16. Carlström K, Döbel A, Pousette A, Rannevik G, Wilking N (1984) Inhibition of steroid sulfatase activity by danazol. *Acta Obstet Gynecol Scand Suppl* 123: 107–111.
17. McGinley R, Casey J (1979) Analysis of progesterone in unextracted serum: A method using danazol — a blocker of steroid binding to proteins. *Steroids* 33: 127–131.