0021-972X/83/5704-0732$02.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 57, No. 4
Printed in U.S.A.

Site of Action of Low Dose Ketoconazole on Androgen Biosynthesis in Men*

R. J. SANTEN, H. VAN DEN BOSSCHE, J. SYMOENS, J. BRUGMANS, AND R. DECOSTER

Department of Medicine, Division of Endocrinology, The Milton S. Hershey Medical Center, The Pennsylvania State University (R.J.S.), Hershey, Pennsylvania 17033; and Janssen Pharmaceutica, Beerse, Belgium

ABSTRACT. Ketoconazole inhibits testosterone biosynthesis in men, but the exact site of its action on the androgen pathway remains to be established. To examine this question, we measured several steroids in the androgen and glucocorticoid pathways in normal men before and after receiving either a single dose of 200 mg ketoconazole or placebo in a cross-over randomized trial. Total and free plasma testosterone fell to levels 60% below basal within 4–8 h ($P < 0.02$ in all) and then returned to control concentrations by 24 h after drug administration. The transient alterations of plasma testosterone correlated well with ketoconazole blood levels, which peaked at 2 h and fell exponentially thereafter. A compensatory increase in plasma LH at 24 h in the drug but not placebo group was consistent with the decrease in plasma testosterone. The levels of plasma androstenedione paralleled those of testosterone in the ketoconazole-treated subjects. In marked contrast, plasma 17α-hydroxyprogesterone increased at 4–8 h (all $P < 0.02$) before returning to basal values at 24 h. This rise in precursor with fall in product steroid implicated an effect of ketoconazole on the C_{17-20} lyase enzyme. This conclusion was supported by the highly significant increase in the ratio of plasma 17α-hydroxyprogesterone to androstenedione observed between 2 and 24 h after drug administration. The effect of ketoconazole at this dose level appeared relatively specific, since no decrements in plasma cortisol or 11-desoxycortisol were found.

During chronic administration of 200 mg ketoconazole daily, decrements of plasma testosterone 2–4 h after drug administration were minimal and documented only by paired comparisons within subjects but not by unpaired tests between normal men and men receiving drug. The lack of major effects on testosterone levels long term at this dosage probably explain why few androgen-related side effects with this drug were previously reported. Ketoconazole, therefore, represents another compound with relatively selective effects on a cytochrome P-450-mediated steroid hydroxylation step, namely that involved with C_{17-20} lyase. (*J Clin Endocrinol Metab* **57:** 732, 1983)

KETOCONAZOLE (Nizoral; Janssen Pharmaceutica, Beerse, Belgium) is an orally active, imidazole derivative used clinically as an antifungal agent (1). This drug inhibits lanosterol conversion to ergosterol in yeast at concentrations equal to or greater than 10^{-9} M, but in mammalian tissue only at concentrations of 10^{-6} M (2, 3). Observations of gynecomastia in a small percentage of men given this drug led to studies of androgen levels after acute administration of ketoconazole (4, 5). A dose-response inhibition of plasma testosterone with single doses from 200–600 mg was found (5). Plasma testosterone decreased maximally to 14% of basal values 8 h after an oral dose of 600 mg ketoconazole. The onset of suppression at 2 h and complete recovery at 24 h paralleled the concentrations of orally administered drug. At the higher doses (*i.e.* 400–600 mg, orally), ketoconazole also blunted the increments in plasma cortisol after ACTH injections (6).

The finding of marked but transient decreases in plasma testosterone acutely and lack of other clinically apparent endocrine effects in men during chronic ketoconazole administration suggested a relatively selective block of steroidogenesis. It appeared pertinent, then, to determine the site of action of ketoconazole on the androgen biosynthetic pathway. We measured the levels of various plasma steroids in normal men to examine precursor to product relationships. This report identifies an effect of ketoconazole in low doses on the cytochrome P-450-dependent C_{17-20} lyase, one of two enzymes that mediate the conversion of 17α-hydroxyprogesterone to androstenedione.

Received March 21, 1983.
Address requests for reprints to: Dr. Richard J. Santen, Department of Medicine, Division of Endocrinology, Pennsylvania State University, Hershey, Pennsylvania 17033.
* Presented in part at the American Society for Clinical Investigation, Washington, D.C., May 8–10, 1982.

Materials and Methods

Patient studies

Experimental protocol: acute. Ten normal men between the ages of 27 and 55 yr volunteered to take a single dose of ketoconazole (200 mg) orally and placebo on another occasion. A randomized

cross-over design was used, with each subject serving as his own control. After a basal sample was drawn at 0900 h, the subjects took either drug or placebo. Subsequent blood samples were collected 2, 4, 8, and 24 h later. After an interval of 1 week, the identical protocol was repeated, but the men were crossed over to receive either drug or placebo, respectively.

Experimental protocol: chronic. Testosterone levels were measured between 2 and 4 h after daily doses of 200 mg ketoconazole. In 10 men (aged 38–70 yr) receiving this drug for onychomycosis, blood samples were obtained basally and at the 3rd, 6th, 9th, and 12th months of ketoconazole administration. Testosterone levels in these patients were compared to those in 10 normal men in whom blood was also collected and processed simultaneously with those in men given ketoconazole.

Assay methods. Plasma 17α-hydroxyprogesterone, androstenedione, and LH concentrations were measured by standard RIA methods used in our laboratories and previously characterized as to specificity, sensitivity, and precision (7–9). The assay for plasma androstenedione used Celite column chromatography before assay, and that for 17α-hydroxyprogesterone used LH-20 chromatography. The remaining methods employed plasma extraction without chromatography (testosterone and 11-desoxycortisol) (10, 11) or direct assay for plasma LH (9) and cortisol (commercial kit assay, CORT-CTK-125 international Cis-Sorin). Free testosterone [free and weakly bound or non-testosterone-estrogen-binding globulin (non-TeBG) bound] was measured with the method of Tremblay and Dube (12), as slightly modified in our laboratory. Ketoconazole levels were measured after purification on high pressure liquid chromatography, as previously described (13).

Statistical methods. Student's paired t tests (two-tailed) were employed to compare values during placebo or drug administration at each time point during acute studies, and unpaired t tests were used for chronic studies.

Results

Acute studies

Plasma testosterone fell from 4.92 ± 0.35 (±SEM) ng/ml to nadir levels of 2.08 ± 0.23 ng/ml 4 h after the administration of drug but not after placebo ($P < 0.01$ *vs.* placebo). Recovery from suppression began at 8 h and was complete at 24 h (Fig. 1A). Free testosterone (*i.e.* non-TeBG bound) fell to a similar extent. These decrements correlated well with drug blood levels, which peaked at 2 h [2.65 ± 0.45 (±SEM) μg/ml) and then fell exponentially at 4 h (1.47 ± 0.32 μg/ml) and 8 h (0.25 ± 0.09 μg/ml). No drug was detectable in plasma at 24 h.

In response to the testosterone decrement, plasma LH increased in the drug- but not in placebo-treated patients (Fig. 1B). There was a high degree of variability in LH measurements, which is attributable to pulsatile LH secretion (9). Nonetheless, at 24 h, plasma LH levels were significantly higher after drug (8.2 ± 0.9 ng/ml) than after placebo (5.6 ± 0.7; $P < 0.01$) administration.

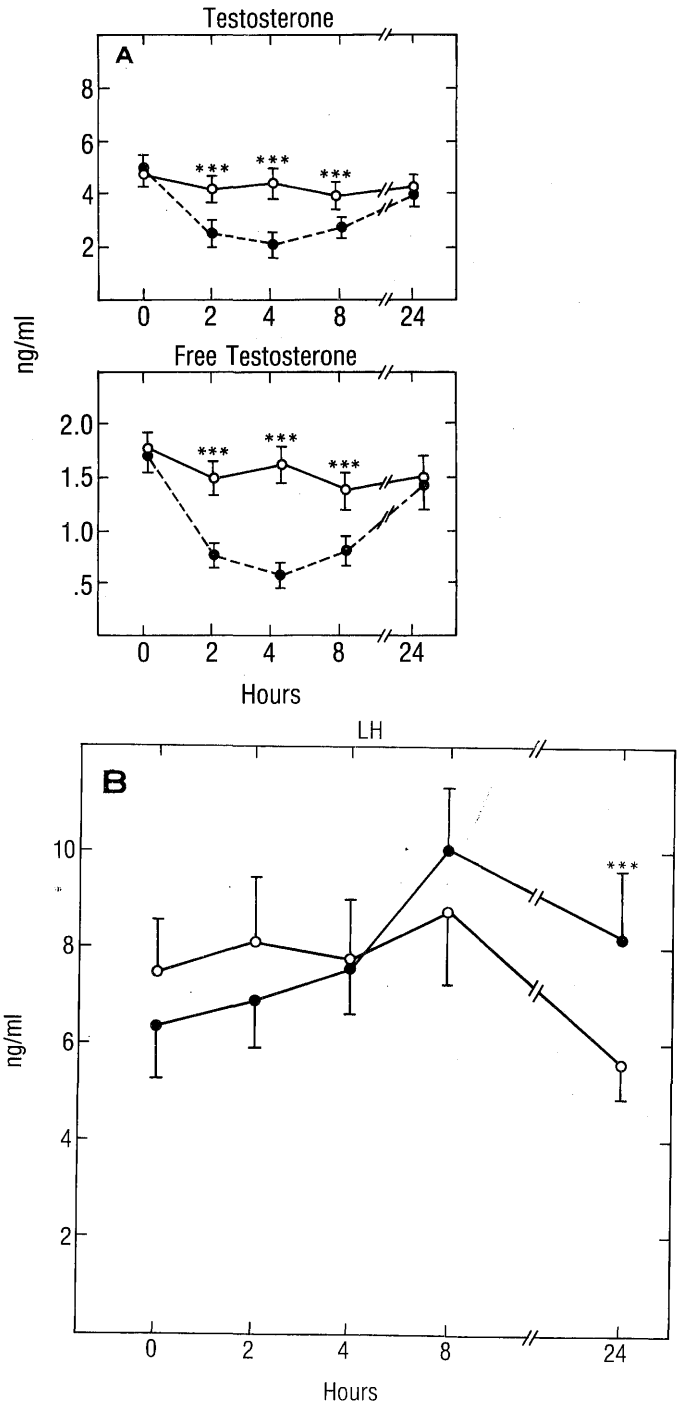

FIG. 1. A, Plasma total and free testosterone levels (mean ± SEM) basally and at various time points after a single 200-mg dose of ketoconazole or placebo given to normal men in a randomized cross-over study (see *Materials and Methods*). *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.01$. O—O, Placebo (n = 10); ●– –●, 200 mg ketoconazole. B, Plasma LH levels. Study design was identical to that in A. O—O, Placebo; ●– –●, 200 mg ketoconazole.

The concentrations of plasma androstenedione paralleled those of testosterone (Fig. 2A), decreasing from 1.0

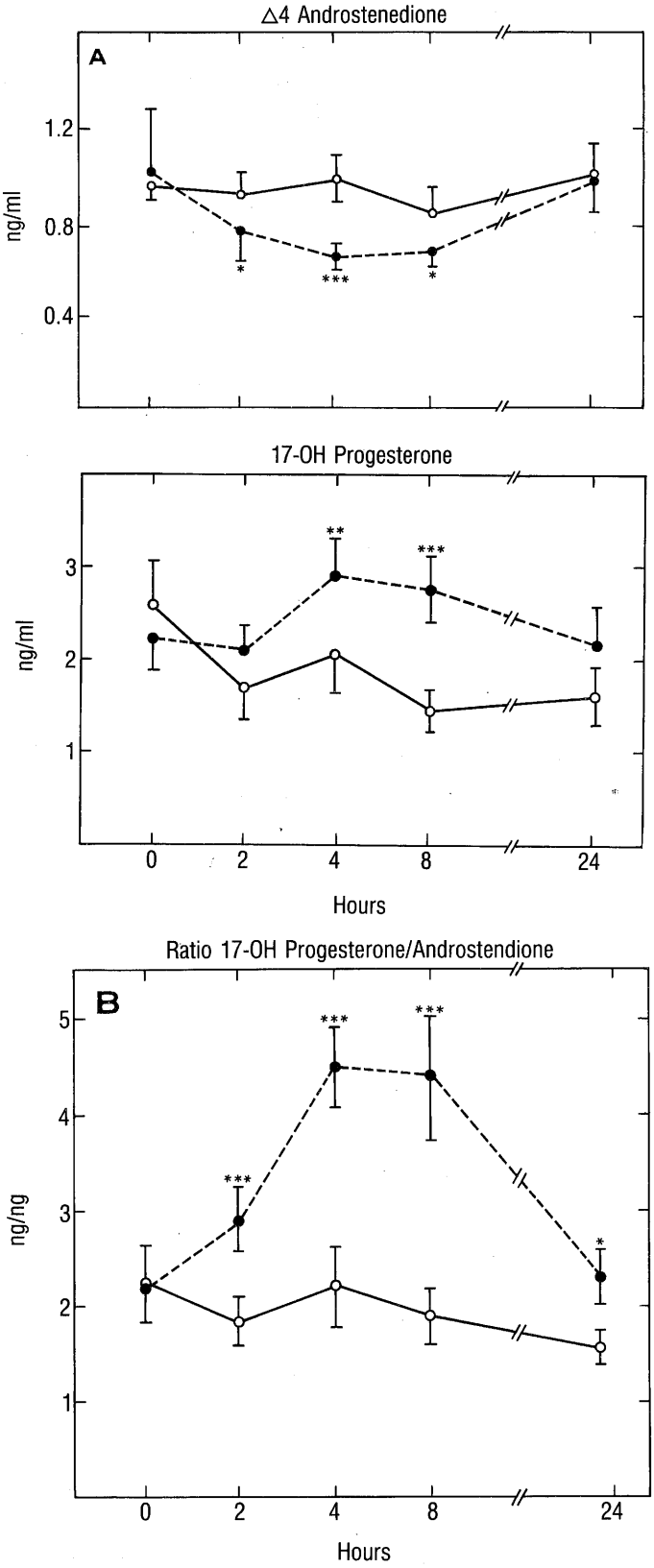

FIG. 2. A, Plasma androstenedione (*top*) and 17α-hydroxyprogesterone (*bottom*) levels. Study design was identical to that in Fig. 1A. ○—○, Placebo; ●– –●, 200 mg ketoconazole. B, Ratios of 17α-hydroxyprogesterone to androstenedione.

± 0.13 to 0.64 ± 0.04 (±SEM) ng/ml ($P < 0.01$) at 0800 h, while recovering at 24 h after drug administration. In marked contrast, the levels of 17α-hydroxyprogesterone increased after drug administration to 2.8 ± 0.4 ng/ml at 8 h, while, after placebo, a diurnal decrement to 1.5 ± 0.2 was found (Fig. 2A). Thus, ketoconazole caused a highly significant increase in 17α-hydroxyprogesterone ($P < 0.01$) at 8 h, with complete return to basal concentrations at 24 h.

The decline in plasma androstenedione and testosterone with an increase in 17α-hydroxyprogesterone suggested a block of the C_{17-20} lyase with accumulation of precursor (17α-hydroxyprogesterone) and a fall in product (androstenedione). To examine this more closely, the ratio of 17α-hydroxyprogesterone to androstenedione was calculated (Fig. 2B). A significant increase in this ratio occurred at 2 h, with a plateau at 4–8 h ($P < 0.001$ and $P < 0.01$ at 4 and 8, respectively) and persistent but minimal increments at 24 h (Fig. 2B).

As a measure of the selectivity of blockade after a single low dose of ketoconazole, plasma cortisol levels were not significantly different at any time point in drug-treated men from those in placebo-treated men. As a result of diurnal changes, cortisol declined by 30% in both groups at 4–8 h and returned to baseline the next morning [placebo: basal, 15 ± 1.3; 4 h, 9.4 ± 1.2; 8 h, 8.4 ± 1.4 μg/dl; ketoconazole: basal, 16.5 ± 1.3; 4 h, 8.0 ± 1.2; 8 h, 9.9 ± 1.5 μg/dl (mean ± SEM)]. 11-Desoxycortisol levels were also similar in both groups of men, except at 8 h when drug-treated men had significantly ($P < 0.01$) higher levels (0.73 ± 0.04 μg/dl) than after placebo treatment (0.63 ± 0.05 μg/dl).

Chronic studies

During chronic therapy with a single daily dose of 200 mg ketoconazole, testosterone levels 2–4 h after the morning dose were slightly lower than basal levels in drug-treated men (Fig. 3). However, nonpaired comparisons of values in normal subjects or in men receiving drug at each time point revealed no statistically significant differences. Drug levels in plasma increased during chronic administration from 0.75 ± 0.15 μg/ml at 3 months to 2.9 ± 0.59 at 12 months. This probably reflects the variable times after drug ingestion (*i.e.* 2–4 h) that the blood samples were collected. This conclusion is supported by an additional study (clinical files of Janssen Pharmaceutica), where plasma ketoconazole levels were constant in five men sampled similarly 2–4 h after morning doses (*i.e.* 3 months, 3.7 ± 0.76 μg/ml; 6 months, 2.9 ± 0.55 μg/ml; 9 months, 2.4 ± 0.53 μg/ml).

Discussion

A wide variety of clinically available drugs exert inhibitory effects on the hypothalamic-pituitary-target gland

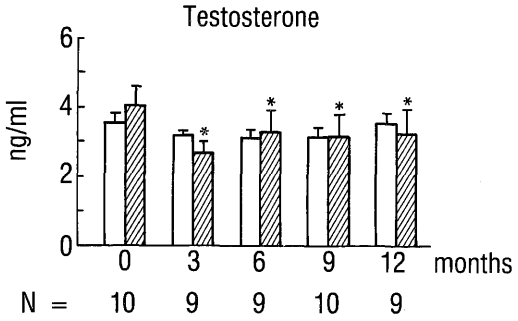

FIG. 3. Plasma testosterone levels during chronic administration of 200 mg ketoconazole as a single oral dose daily in 10 patients (▨) and in 10 normal men (▭). All blood samples were collected 2–4 h after single doses of drug in the patients and at the same time in the normal subjects. *, $P < 0.05$, basal *vs.* treatment. The differences between normal and drug-treated patients were not significantly different at any time point.

axes. Certain of these compounds inhibit the biosynthesis of steroids involved in negative feedback control systems. Compensatory increments of the respective trophic hormones result, which can attenuate or completely mask the endocrine inhibitory properties of these drugs. For this reason, subtle endocrine actions of certain drugs have not been detected in basic toxicological, preclinical, and early clinical testing. Only after exposure of large numbers of patients have definite and often potent endocrinological effects of certain compounds been noted. For example, the potent adrenal inhibitory and T_4-blocking properties of aminoglutethimide were undetected during early testing and clinical introduction (14). Because of compensatory ACTH and TSH increments occurring during aminoglutethimide administration, the majority of subjects initially receiving this compound experienced no signs or symptoms of cortisol or T_4 deficiency (15, 16).

Ketoconazole, similarly, produced no effects on the male reproductive tract or symptoms of androgen deficiency in preclinical and early clinical trials (17–20). Reports of gynecomastia in a limited number of patients receiving larger doses led to initial endocrinological studies (4, 5). The decrements in testosterone and compensatory increments in plasma LH observed in this and earlier studies (5) demonstrated a potent effect of ketoconazole on androgen biosynthesis. Two factors minimize the significance of this effect on a practical basis. First, the drug is given as a single daily dose, and the half-life is short (*i.e.* 4 h). Consequently, the acute reduction of testosterone is transient, and complete recovery in androgen levels occurs by 24 h. Secondly, a compensatory increase in LH results, which, analogous to the ACTH increment after aminoglutethimide treatment, may allow the effect of low doses of drug to be partially or completely overcome during chronic administration (5). This latter effect may explain why plasma testosterone levels were minimally suppressed during daily administration of 200 mg, orally, for up to 12 months. Unfortunately, additional plasma for LH, FSH, and estradiol assays is not currently available for further assessment of the drug effects during chronic (or acute) administration.

Doses of ketoconazole higher than 200 mg daily, when administered chronically, may persistently suppress androgen production in men (Dunn, J., and J. Graybill, presented at a workshop meeting on ketoconazole at Janssen Pharmaceutica, Beersa, Belgium, January 22, 1983). Pont *et al.* (5) found greater suppression of testosterone with a single 600-mg dose than with 200 mg. This could be due in part to additional blocking effects of ketoconazole. For example, enzymes in addition to the C_{17-20} lyase could be inhibited with higher ketoconazole doses. Cortisol responsiveness to ACTH is blunted during the administration of 400–600 mg ketoconazole (6). Such pharmacological effects would be analogous to those observed with aminoglutethimide which blocks aromatase at low doses (*i.e.* 0.3 μM), and also cholesterol side-chain cleavage and 18-hydroxylation of corticosterone at higher concentrations (3 μM) (14, 21). These considerations indicate that further dose-response studies of ketoconazole on various steroid biosynthetic enzymes are now required.

Inferences regarding the site of action of drugs such as ketoconazole with measurements of circulating plasma levels of steroid are indirect. Alterations of steroid MCRs, for example, can perturb the ratios of precursor to product in plasma independent of an enzymatic block. However, a change in the androstenedione or 17-hydroxyprogesterone MCRs within 4–8 h after the administration of a single dose of ketoconazole would not be expected. The drug effect on plasma steroid ratios could also be explained on another basis. A greater release of steroid precursor from its site of synthesis and a lesser leak of product from tissue without an associated enzymatic blockade could explain altered precursor to product steroid ratios. This possibility is unlikely, since *in vitro* ^{3}H-labeled steroid metabolic studies also demonstrated an inhibitory effect of ketoconazole on the conversion of 17α-hydroxyprogesterone to androstenedione (H. Van den Bossche, H., presented at a workshop conference on ketoconazole at Janssen Pharmaceutica, Beersa, Belgium, January 23, 1983).

Androstenedione and 17α-hydroxyprogesterone originate from both adrenal and gonadal sources in men. Ketoconazole could block the testis or the adrenal exclusively, or both glands simultaneously. The fall in plasma testosterone, a nearly exclusive testicular steroid in men, suggests a direct gonadal effect. An additional action on the adrenal is suggested by the blunting of ACTH-stimulated cortisol during the administration of higher doses

of ketoconazole (6). Whether a single 200-mg dose blocks adrenal C_{17-20} lyase as well as its testicular counterpart remains to be established.

It would provide additional support for our conclusions to demonstrate that steroid levels in pubertal boys with congenital C_{17-20} lyase deficiency are similar to the concentrations in our patients. Zachmann *et al.* (22) recently reported 3 patients with this congenital defect and reviewed the literature in 11 others. In all 3 patients, the 17α-hydroxyprogesterone levels were only slightly elevated and increased further after hCG or ACTH administration. The degree of inhibition of androstenedione and testosterone biosynthesis varied from mild in two patients to profound in the third. Taken together, the degree of changes in 17α-hydroxyprogesterone, androstenedione, and testosterone in Zackmann's patients are similar to those 4–8 h after a single dose of ketoconazole.

Zackmann *et al.* (22) speculated that two enzymes exist for the conversion of 17α-hydroxyprogesterone to androstenedione. The first enzyme is involved in the conversion of both 17α-hydroxypregnenolone to dehydroepiandrosterone (Δ^5 pathway) and 17α-hydroxyprogesterone to androstenedione (Δ^4 pathway), and the second (the C_{17-20} desmolase, strictore sensu) exclusively mediates the Δ^4 pathway conversion. *In vitro* studies to be published separately by Van den Bossche suggest that ketoconazole inhibits the latter of these two enzymes, which mediates the conversion of 17α,20α-dihydroxyprogesterone to androstenedione. Studies to determine the effects of ketoconazole on the Δ^5 pathway *in vitro* are ongoing. This methodological approach will allow evaluation of these effects without the confounding action of LH in producing a switch from the Δ^4 to the Δ^5 pathway (23).

In summary, ketoconazole blocked C_{17-20} lyase and caused an acute rise in 17α-hydroxyprogesterone, with decrements in androstenedione and testosterone. This effect induced a compensatory rise in LH secretion. During chronic administration of 200 mg ketoconazole as a single daily dose, testosterone levels were not suppressed, perhaps as a result of persistent mild increments in LH secretion.

Acknowledgments

The authors are grateful to Mr. R. Woestenbroghs for determination of ketoconazole plasma levels. Mrs. Marlene Thompson provided excellent secretarial assistance. The authors acknowledge the support of the Core Endocrine Laboratory for assistance with the RIA measurements.

References

1. Graybill JR, Drutz DJ 1980 Ketoconazole: a major innovation for treatment of fungal disease. Ann Intern Med 93:921
2. Van den Bossche H, Willemsens G, Cools W, Cornelissen F, Lauwers W, Cutsem J 1980 *In vitro* and *in vivo* effects of the antimycotic drug ketoconazole on sterol synthesis. Antimicrob Agents Chemother 17:922
3. Willemsens G, Cools W, Van den Bossche H 1980 Effects of miconazole and ketoconazole on sterol synthesis in a subcellular fraction of yeast and mammalian cells. In: Van den Bossche H (ed) The Host Invader Interplay. Elsevier North Holland, New York, pp 691–694
4. DeFelice R, Johnson DG, Galgiani JN 81 Gynecomastia with ketoconazole. Antimicrob Agents Chemother 19:1073
5. Pont A, Williams PL, Azhar S, Reitz RE, Bochra C, Smith ER, Stevens DA 1982 Ketoconazole blocks testosterone synthesis. Arch Intern Med 142:2137
6. Pont A, Williams PL, Loose DS, Feldman D, Reitz RE, Bochra C, Stevens DA 1982 Ketoconazole blocks adrenal steroid synthesis. Ann Intern Med 97:370
7. Samojlik E, Santen RJ 1978 Adrenal suppression with aminoglutethimide. III. Comparison of plasma Δ^4-Δ^5-steroids in postmenopausal women treated for breast carcinoma. J Clin Endocrinol Metab 47:717
8. Samojlik E, Santen RJ, Wells SA 1977 Adrenal suppression with aminoglutethimide. II. Differential effects of aminoglutethimide on plasma androstenedione and estrogen levels. J Clin Endocrinol Metab 45:480
9. Santen RJ, Bardin CW 1973 Episodic luteinizing hormone secretion in man. Pulse analysis, clinical interpretation, physiological mechanisms. J Clin Invest 52:2617
10. De Coster R, Beckers JF, Wouters-Ballman P, Ectors F 1979 Variations nycthemerales de la testosterone et de la lutropine plasmatiques chez le chien. Ann Med Vet 123:423
11. Demers LM, Ebright L, Derck DD 1979 Improved radioimmunoassay for 11-deoxycortisol (compound S) in plasma. Clin Chem 25:1704
12. Tremblay RR, Dube JY 1974 Plasma concentrations of free and non-TeBG bound testosterone in isomers of oral contraceptives. Contraception 10:599
13. Woestenbroghs R, Embrechts L, Heykants J 1982 HPLC Method for the Specific Determination of Ketoconazole in Plasma. Preclinical Research Report R41 400/34, Janssen Pharmaceutica, Beerse, Belgium
14. Santen RJ, Henderson IC 1981 Pharmanual: A Comprehensive Guide to the Therapeutic Use of Aminoglutethimide. Karger, Basel and New York
15. Santen RJ, Wells SA, Cohn N, Demers LM, Misbin RI, Foltz EL 1977 Compensatory increase in TSH secretion without effect on prolactin secretion in patients treated with aminoglutethimide. J Clin Endocrinol Metab 45:739
16. Fishman LM, Liddle GW, Island DP, Fleischer N, Kuchel O 1967 Effects of aminoglutethimide on adrenal function in man. J Clin Endocrinol Metab 27:481
17. Restrepo A, Stevens DA, Utz JP 1980 First International Symposium on Ketoconazole. Rev Infect Dis 2:519
18. Stevens DA, Stiller RL, Williams PL, Sugar AM 1982 Experience with ketoconazole in three major presentations of progressive coccidioidomycosis. Am J Med 74:58
19. Levine HB 1982 Ketoconazole in the Management of Fungal Disease. ADIS Press, New York
20. Graybill JR 1983 Introduction: an update on progress with ketoconazole. Am J Med 74:1
21. Santen RJ, Santner S, Davis B, Veldhuis J, Samojlik E, Ruby E 1978 Aminoglutethimide inhibits extraglandular estrogen production in postmenopausal women with breast carcinoma. J Clin Endocrinol Metab 47:1257
22. Zachmann M, Werder EA, Prader A 1982 Two types of male pseudohermaphroditism due to 17,20-desmolase deficiency. J Clin Endocrinol Metab 55:487
23. Glass AR, Vigersky RA 1980 Resensitization of testosterone production in men after human chorionic gonadotropin induced desensitization. J Clin Endocrinol Metab 55:487

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.