[CANCER RESEARCH 53, 4563–4566, October 1, 1993]

Inhibition of the *in Vivo* Conversion of Androstenedione to Estrone by the Aromatase Inhibitor Vorozole in Healthy Postmenopausal Women[1]

Elsken van der Wall, Truus H. Donker, Edwin de Frankrijker, Hans W. R. Nortier, Jos H. H. Thijssen,[2] and Marinus A. Blankenstein

Department of Endocrinology, Academic Hospital Utrecht, 3508 GA Utrecht [T. H. D., E. F., J. H. H. T., M. A. B.], and Department of Internal Medicine, Diakonessen Hospital Utrecht, 3582 KE Utrecht [E. W., H. W. R. N.], the Netherlands

ABSTRACT

Vorozole is a new, potent, and highly selective nonsteroidal aromatase inhibitor, which in animal and human studies was found to be about 1000-fold more potent than aminoglutethimide. Almost all aromatase-inhibiting activity resides in the dextro-enantiomer currently undergoing clinical trials. A marked decrease in circulating estrogens was found in several studies of healthy premenopausal women and male volunteers treated with the racemate, referred to as vorozole racemate.

To further evaluate the aromatase-inhibiting potency of this drug, the *in vivo* conversion of androstenedione to estrone was studied in 12 healthy postmenopausal women. Four h after a single oral dose of vorozole racemate, [^{14}C]androstenedione and [^{3}H]estrone were infused at a constant rate for 2 h. Women were randomized to receive vorozole racemate orally in one of three different doses, *i.e.*, 1, 2.5, and 5 mg, in a double-blind protocol. Each woman acted as her own control in an identical experiment with a placebo carried out 2–4 weeks either before or after the test with vorozole racemate. In the urine, collected for 4 days after each experiment, estrogens were extracted and purified until a constant ^{3}H/^{14}C ratio of estrone was achieved.

The percentage conversion of androstenedione to estrone in the 12 placebo experiments was 2.19 ± 0.60% (mean ± SD, n = 12). Following a single administration of vorozole racemate, the conversion decreased to 0.14 ± 0.04%. The percentage inhibition was 93.0 ± 2.5 (n = 4) following administration of 1 mg vorozole racemate; administration of 2.5 or 5 mg resulted in an inhibition percentage of 93.2 ± 1.6 or 94.4 ± 1.2, respectively.

It is concluded that a single oral dose of 1–5 mg vorozole racemate results in an almost complete inhibition of *in vivo* aromatase activity.

INTRODUCTION

Estrogens are known to promote the growth of breast carcinoma (1), and inhibition of the growth-stimulating activity of estrogens is one of the major aims in the treatment of this tumor (2). This can be achieved either by preventing the interaction between estrogens and their receptors within the tumor cell by antiestrogens or by interfering with the production of estrogens.

The major pathway for estrogen biosynthesis is the aromatization of androgens (3) by the aromatase, a cytochrome P450-regulated enzyme complex which is responsible for the conversion of the androgens testosterone and androstenedione to estrone and estradiol (4, 5). In premenopausal women, aromatase activity is mainly found in the ovaries. In postmenopausal women, however, the main source of circulating estrogens is the peripheral aromatization of mainly androstenedione in adipose tissues, including the breast (6, 7).

Aminoglutethimide was the first aromatase inhibitor to be used in the treatment of metastatic breast cancer in postmenopausal women (8–10). Its effectiveness in lowering the plasma levels of circulating estrogens equals the reduction seen after surgical adrenalectomy (11). The concomitant inhibition of the cholesterol side-chain cleavage, resulting in the inhibition of adrenal steroidogenesis, however, necessitates the concurrent administration of glucocorticoids (12). In addition, moderate side effects are observed (13). More recently, newer and more specific aromatase inhibitors, such as 4-hydroxyandrostenedione (14–16) and fadrozole (17–20), were developed and were used successfully in the treatment of metastatic breast carcinoma in postmenopausal women.

Another new nonsteroidal aromatase inhibitor, Vorozole, has recently been developed (21–27). *In vitro* and *in vivo* studies in animal models demonstrated a high potency and specificity and, thus, its potential clinical usefulness in humans. Almost all aromatase activity resides in the dextro-enantiomer (R83842) of a racemic mixture (R76713), referred to as vorozole racemate (21, 28). Studies with vorozole racemate in healthy male volunteers showed a marked decrease in serum estradiol levels. The drug was very well tolerated, and with doses of up to 20 mg vorozole racemate, no consistent changes in hematological and biochemical parameters were observed (23). In an ongoing phase I study, postmenopausal women with advanced breast cancer showing clinical progression during treatment with tamoxifen were randomized to receive 2.5 or 5 mg vorozole racemate. Evaluation after 3 months showed objective (regression of bone metastasis and skin metastasis) and subjective (decrease on the World Health Organization pain and Karnofsky scales) responses. Estradiol levels decreased to the detection limit of the assay (10 pmol/liter) during treatment (29).

The main purpose of the present study was to establish the effect of vorozole racemate on the *in vivo* conversion of androstenedione to estrone. In 12 healthy postmenopausal women, the *in vivo* aromatase activity was measured by determining the cumulative ^{3}H/^{14}C ratio of estrone in the urine after infusion of [^{3}H]estrone and [^{14}C]androstenedione before and after administration of different doses of vorozole racemate.

SUBJECTS AND METHODS

Subjects. Twelve healthy postmenopausal women participated in this study after informed consent. The exclusion criteria used were: time from last menstrual period <2 years, younger than 55 years after hysterectomy, hormonal treatment for any reason within the last 2 months, endocrinological disorders including diabetes mellitus, diseases of the gastrointestinal tract which would interfere with the absorption of vorozole racemate, and signs of liver and/or renal malfunctioning as reflected in biochemical parameters. The study was approved by the medical ethical committee of the Academic Hospital, Utrecht, The Netherlands. The participating women were 65 ± 4 (mean ± SD) years of age, had a height of 1.67 ± 0.09 m, and had a weight of 64 ± 7 kg. All had FSH[3] levels of >55 IU/liter (74 ± 16 IU/liter) and estradiol levels <55 pmol/liter (22 ± 13 pmol/liter), well in the postmenopausal ranges. Thyroid and adrenal function were clearly within the reference ranges.

The 12 women were randomized into three groups according to the different doses of vorozole racemate: 1, 2.5, and 5 mg. Each of the participants served as her own control in an identical experiment with a placebo, carried out 2–4

Received 4/7/93; accepted 7/28/93.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Preliminary results of this study were presented at the VIII International Congress on Hormonal Steroids, The Hague, the Netherlands, September 1990.

[2] To whom requests for reprints should be addressed, at Department of Endocrinology, Academic Hospital G02.625, PO Box 85500, 3508 GA Utrecht, the Netherlands.

[3] The abbreviations used are: FSH, follicle-stimulating hormone; MCR, the metabolic clearance rate.

weeks either before or after the test with the active drug. The sequence of administration was unknown to the investigators as well as to the women.

Administration of the Drug. Vorozole (6-[(4-chlorophenyl)(1H-1,2,4-triazol-1-yl)methyl]-1-*methyl*-1H-benzotriazole) was synthesized at the Chemical Research Centre of the Janssen Research Foundation (Beerse, Belgium). The dextro-isomer, R83842 (Vorozole), accounts for the aromatase inhibitory activity almost exclusively (28). The present study was performed using the racemic mixture (R76713), referred to as vorozole racemate. The racemate as well as the placebo were dissolved in propylene glycol (145 μl) and hydroxypropylbetacyclodextrine (50 mg). Water was added to make a total volume of 1 ml. Just prior to administration, about 50 ml tap water was added. The drug was tolerated very well. Two women complained of mild drowsiness for approximately 4 days after one of the infusion experiments; the experiments concerned appeared to be the placebo experiments.

Experimental Procedures. Approximately 15 μCi [^{14}C]androstenedione and 15 μCi [^{3}H]Estrone (Amersham International, Buckinghamshire, United Kingdom) were dissolved in 25 ml sterile saline, containing 5% ethanol, using experimental procedures described previously (30). One-third of the radioactive steroid solution was given i.v. as a priming dose at 3.5 h after a single p.o. dose of vorozole racemate or the placebo; the remainder was infused at a constant rate over 2 h, starting 30 min after the priming dose. The amount of labeled hormone administered accounted for <5% of the endogenous production of androstenedione and estrone.

Blood samples to determine plasma levels of endogenous hormones were obtained 15 min before administration of the priming dose. Blood samples at 15 min before and at the end of the infusion were used to measure the MCRs (30). The MCR and the levels of the endogenous steroids were used to calculate the blood production rates of androstenedione and estrone. From the start of the experiments, the participants were asked to collect their urine four times during 24 h. The excretion of radioactivity in the daily urine samples was determined by counting the concentration of both radioactive labels in triplicate. No attempt was made to quantitate the fecal excretion of steroid metabolites.

In the urine, the ^{3}H/^{14}C ratio of estrone was measured after its purification and derivatization until a constant ratio was obtained: 10% portions of the daily urine samples were mixed and two 100-ml samples of this urine mixture were used for duplicate analysis.

Briefly, the procedure used was as follows. To each portion of urine, 10 ml of 0.1 M acetate buffer (pH 4.7) and 0.6 ml of Suc d'Helix Pomatia (Sepracor S. A., Villeneuve-la-Garenne, France) were added. Hydrolysis was carried out during 24–48 h at 37°C. Subsequently, the urine was transferred to an activated 6-ml C_{18} column (Baker, Deventer, the Netherlands) and rinsed with water and 20% methanol; finally, steroids were eluted with 100% methanol, and the eluate was evaporated. The residue was dissolved in 0.2 ml of 100% methanol, and 5 ml of petroleum ether (40–60°C)-1:1 (v/v) toluene were added. Extraction with 5 ml of water was followed by extraction of the estrogens with 5 ml of 0.4 N NaOH. The alkaline extract was adjusted to pH 9 with 0.3 g of $NaHCO_3$, and the resulting solution was extracted 3 times with 4 ml of diethylether. The combined ether extracts were evaporated under a stream of nitrogen and dissolved in 0.25 ml of toluene:methanol (92:8). Estrone was separated from estradiol by chromatography on a Sephadex LH-20 column using toluene:methanol (92:8) as the eluate. The fraction containing estrone was collected, evaporated, and purified to a constant ^{3}H/^{14}C ratio by consecutive derivatization steps, *i.e.*, acetylation, hydrolysis, methylation at C3, reduction at C17, and finally acetylation at C17, each derivative being subjected to chromatographic purification (31).

The percentage conversion of androstenedione to estrone was calculated from the constant ^{3}H/^{14}C ratio in the final three derivatives using the formula:

$$\% \text{ Conversion} = \frac{^{3}\text{H}/^{14}\text{C administered steroids}}{^{3}\text{H}^{14}\text{C urinary estrone}} \times 100\%.$$

Hormone Measurements. FSH was determined using the Enzymun-Test from Boehringer (Mannheim, Germany), and values are expressed in IU 1st International Reference Preparation of FSH; details were described previously (32). Androstenedione and testosterone were measured after extraction from the plasma (33), and estradiol and estrone were measured after extraction and LH-20 column chromatography (33).

Statistical Analysis. The significance of differences between results obtained from the same woman was evaluated using paired Wilcoxon's rank sum test; significance of differences between different groups were evaluated using the two-sample Wilcoxon rank sum test.

RESULTS

Excretion of Radioactive Metabolites. One of the requirements for measurements of *in vivo* conversion rates is a practically complete excretion of the urinary metabolites. After 4 days, the average total urinary excretion was 52 ± 9% of the metabolites of [^{3}H]estrone (n = 24) and 74 ± 7% of [^{14}C]androstenedione (n = 24). On the final day of collection, <10% of the urinary excretion was measured. In Fig. 1 the excretion of radioactivity after the placebo and vorozole racemate experiments are given separately. From these results it is clear that there were no differences between the placebo and the active drug experiments in the amount and the daily excretion rates of the metabolites.

Steroid Levels and Production Rates. Because of a technical laboratory problem, not all MCR values for estrone following the administration of vorozole racemate are available. The remaining values of the MCR and the corresponding blood production rates for androstenedione and estrone are presented in Table 1. The values obtained are well within reference values for postmenopausal women (30). Between the two experiments, no differences were found in the metabolic clearance rates of the steroids under investigation. The calculated blood production rates in Table 1 are based on hormone levels measured soon after the administration of the active compound. The expected decrease in plasma estrone after vorozole racemate has not yet taken place and thus will not effect the calculations. The testosterone blood concentrations during the placebo experiment were 0.77 ± 0.26 nmol/liter (n = 12) compared with 0.86 ± 0.24 nmol/liter (n = 12) just after vorozole racemate administration; estradiol levels were 22 ± 12 and 23 ± 14 pmol/liter, respectively.

***In Vivo* Aromatization.** During the placebo experiment 2.19 ± 0.60% (range, 1.23–3.09%; n = 12) of the administered androstenedione was aromatized to estrone, again well within normal limits for women of this age. The three groups of women, divided according to the dose of vorozole racemate, did not show significant differences in percentages of conversion after placebo treatment. Following administration of vorozole racemate, the conversion decreased to 0.14 ±

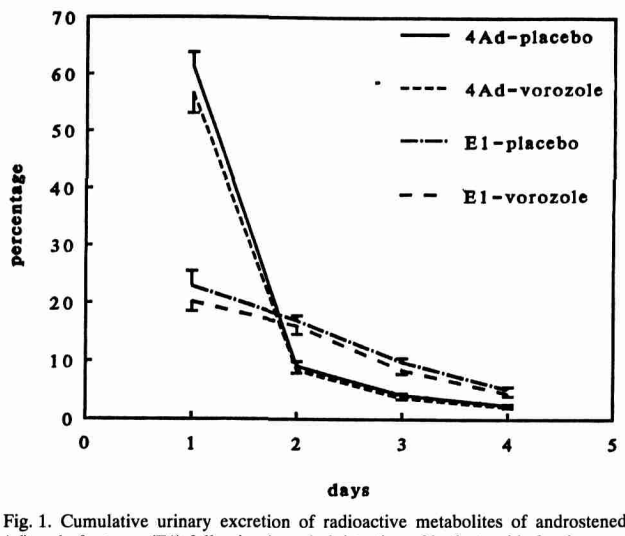

Fig. 1. Cumulative urinary excretion of radioactive metabolites of androstenedione (4-Ad) and of estrone (E1) following i.v. administration of both steroids for the measurements of metabolic clearance rates. The excretion is expressed as a percentage of the total amount of radioactive steroid given to each individual. Results obtained after placebo experiments and with vorozole racemate are shown separately. The days were counted from the time of administration of the steroids. *Point* (*bar*), mean ± SEM.

Table 1 *Effect of administration of placebo or of 1, 2.5, or 5 mg of vorozole racemate on the MCR of androstenedione (4-Adion) and estrone (E1) in normal postmenopausal women*

Vorozole racemate was administered orally 4 h before the clearance rate measurements. All results following the active compound have been combined; no differences were found between the two experiments.

	MCR (liters/24 h)		Concentration		Production rate	
	4-Adion	E1	4-Adion (μg/liter)	E1 (ng/liter)	4-Adion (mg/24 h)	E1 (μg/24 h)
Total mean	1970	2482	0.51	18.7	1.00	43.2
SD	459	606	0.32	6.9	0.62	17.2
SEM	100	147	0.07	1.4	0.14	4.2
N	22	18	24	24	22	18
Placebo mean	2112	2324	0.43	19.0	0.87	44.1
SD	443	548	0.20	6.1	0.35	18.2
SEM	133	165	0.06	1.9	0.11	5.5
N	12	12	12	12	12	12
Vorozole mean	1800	2799	0.59	18.3	1.17	41.6
SD	419	591	0.42	8.3	0.81	15.1
SEM	140	264	0.13	2.5	0.27	6.7
N	10	6	12	12	10	6

0.04% (range, 0.08–0.21%; $n = 12$). The percentage of inhibition of the aromatization caused by the three different doses of vorozole racemate compared to placebo is shown in Fig. 2; the placebo conversion rates are given as 100% in each group.

Administration of 1 mg vorozole racemate resulted in an inhibition of the conversion of 93.0 ± 1.6%. Following ingestion of 2.5 or 5 mg vorozole racemate, very similar inhibition percentages of 93.2 ± 1.6 and 94.4 ± 1.2, respectively, were observed. This justifies the conclusion that a single oral dose of 1 to 5 mg vorozole racemate results in an almost complete inhibition of the *in vivo* aromatase activity.

DISCUSSION

In previous studies with Vorozole in animals and in healthy males, the effectiveness of this aromatase inhibitor was demonstrated *in vitro* as well as *in vivo*. Vorozole is an excellent inhibitor specifically of the aromatase cytochrome P-450, as demonstrated by *in vitro* studies (21–24, 25, 27). The *in vivo* studies (29) were based on its potency to decrease estrogen levels in the circulation. The ultimate proof of inhibition of the aromatase system is the demonstration of an effect on the peripheral aromatization of androstenedione, the main source of estrogens in postmenopausal women.

This study extends the observations *in vivo* to postmenopausal women. A very low dose of the racemate and thus of Vorozole is effective in decreasing the biosynthesis of estrogens in postmenopausal women. The high activity of Vorozole is indicated by the fact that 1 mg of the racemate inhibits the aromatase almost completely. The racemic mixture (R76713) was used in the present study. Because almost all aromatase-inhibiting activity resides in the dextro-enantiomer, extrapolation of the observed activity to the active component is justified.

No effect of vorozole racemate on the metabolism of the infused steroids was found in the women studied. The daily excretion of urinary metabolites and the metabolic clearance rates of the steroids under investigation were highly comparable during the experiments after administration of vorozole racemate or the placebo. Total urinary excretion accounts for <100% recovery because a substantial amount

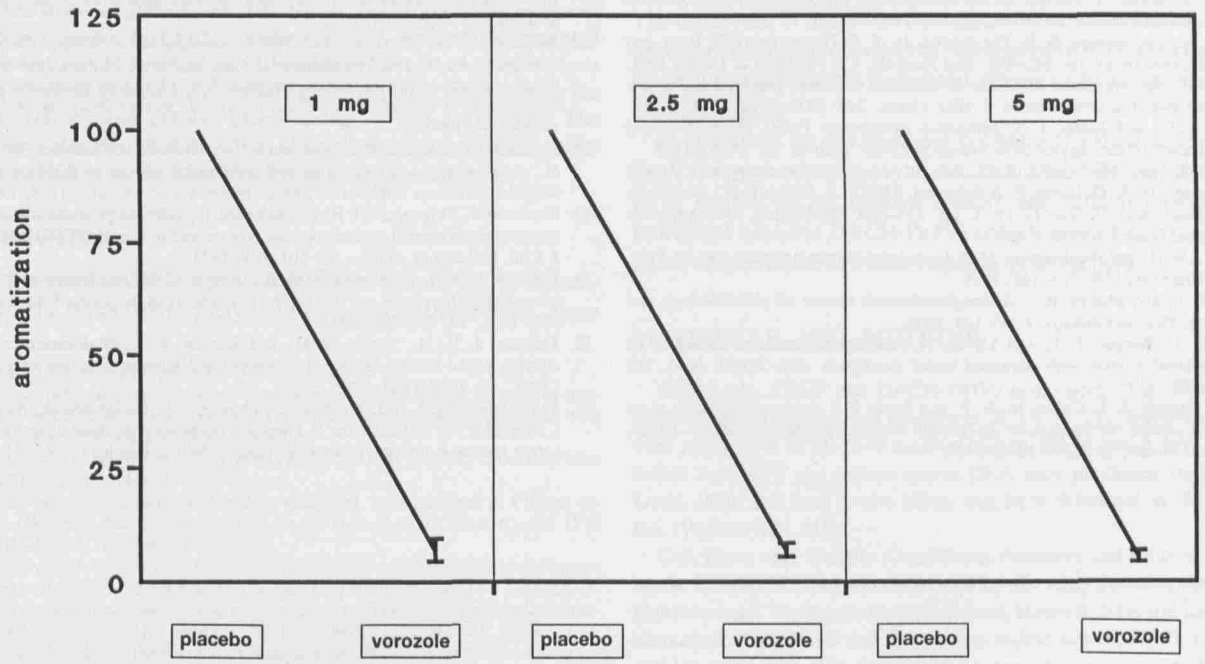

Fig. 2. Effect of different doses of vorozole racemate on the *in vivo* peripheral aromatization in normal postmenopausal women. The percentage of conversion during the placebo experiment in the same woman was taken as 100%. After administration of the active compound, the conversion decreased an average of 94%.

of metabolites will be excreted via the gastrointestinal tract. The fecal excretion does not affect the calculation of conversion rates based on urinary estrone.

The mean blood production rate of estrone (Table 1) is higher than the calculated amounts of estrone derived from androstenedione. This is in contrast with the well-established fact that the major source of estrone in postmenopausal women is peripheral formation from androstenedione (6). The explanation for this discrepancy must be sought in the principles of the techniques used. Peripheral conversion rates are based correctly on measurements of urinary estrone; they give information concerning the conversion rates over many hours, whereas blood production rates in this study are based on single measurements of the MCR and blood hormone levels in the afternoon and late morning, respectively. These parameters show substantial variations over the day, and they are influenced by posture.

The main aim of this study was to show inhibition of the peripheral conversion after Vorozole treatment. Because the urinary-based conversion rates measure total conversion, the large reduction in this conversion proves that the aromatization is inhibited to a large extent. Thus, the compound looks very promising, and indeed, the first clinical trials indicate its effectiveness (29). More trials will be needed to establish its real value in treatment of patients with breast cancer.

As previously documented, local aromatase activity in human breast tissues may play a role in providing small quantities of estrogens involved in growth stimulation of malignant breast tumors (7, 31). Although Vorozole is highly effective in inhibiting the aromatase *in vitro*, no evidence is available concerning an *in vivo* effect of Vorozole on estradiol locally in the human breast. Studies to establish such an effect are currently being undertaken.

ACKNOWLEDGMENTS

The authors are grateful to Dr. R. De Coster, Janssen Research Foundation, Beerse, B 2340 Belgium, for supply of the active components plus placebo and for valuable suggestions for the manuscript.

REFERENCES

1. Mühlblock, O. Role of hormones in the etiology of breast cancer. J. Natl. Cancer Inst., *48:* 1213–1220, 1972.
2. Santen, R. J., Manni, A., Harvey, H., and Redmond, C. Endocrine treatment of breast cancer in women. Endocr. Rev., *11:* 221–265, 1990.
3. Goldfien, A., and Monroe, S. E. The ovaries. *In:* F. S. Greenspan (ed.), Basic and Clinical Endocrinology, pp. 442–490. East Norwalk, CT: Appleton & Lange, 1991.
4. Thompson, E. A., and Siiteri, P. K. The involvement of human placental microsome cytochrome P-450 in aromatization. J. Biol. Chem., *249:* 5373–5378, 1974.
5. Vickery, L. E., and Kellis, J. T. Aromatase cytochrome P-450. Purification and characterization of the enzyme from human placenta. Steroids, *50:* 29–36, 1987.
6. Siiteri, P. K., and MacDonald, P. C. Role of extraglandular estrogen in human endocrinology. *In:* R. O. Greep, E. B. Astwood, AND S. R. Geiger (eds.), Handbook of Physiology, Sect. 7, Vol. II, Pt. 1, pp. 615–629. Washington, DC: American Physiological Society, 1973.
7. Thijssen, J. H. H., and Blankenstein, M. A. Oestrogens in breast tumours and fat. Proc. R. Soc. Edinburgh, *95B:* 161–168, 1989.
8. Santen, R. J., and Misbin, R. I. Aminoglutethimide: review of pharmacology and clinical use. Pharmacotherapy, *1:* 95–120, 1981.
9. Santen, R. J., Worgul, T. J., and Lipton, A. Aminoglutethimide as treatment of postmenopausal women with advanced breast carcinoma. Ann. Intern. Med., *96:* 94–101, 1982.
10. Cash, R., Brough, A. J., Cohen, M. N. P., and Satoh, P. S. Aminoglutethimide as an inhibitor of adrenal steroidogenesis: mechanism of action and therapeutic trial. J. Clin. Endocrinol. Metab., *27:* 1239–1248, 1967.
11. Santen, R. J., Wargul, T. J., Samojlik, E., Interrante, A., Boucher, A. E., Lipton, A., Harvey, H. A., White, D. S., Smart, E., Cox, C., and Wells, S. A. A randomized trial comparing surgical adrenalectomy with aminoglutethimide plus hydrocortisone in women with advanced breast cancer. N. Engl. J. Med., *305:* 545–551, 1981.
12. Santen, R. J., Lipton, A., and Kendall, J. Successful medical adrenalectomy with aminoglutethimide. Role of altered drug metabolism. J. Am. Med. Assoc., *230:* 1661–1665, 1974.
13. Harris, A. L., Dowsett, M., Jeffcoate, S. L., and Smith, I. E. Aminoglutethimide dose and hormone suppression in advanced breast cancer. Eur. J. Cancer Clin. Oncol., *19:* 493–498, 1983.
14. Brodie, A. M. H., and Wing, L. Y. *In vitro* and *in vivo* studies with aromatase inhibitor 4-hydroxy-androstenedione. Steroids, *50:* 89–103, 1987.
15. Coombes, R. C., Goss, P. E., Dowsett, M., Hutchinson, G., Cunningham, D., Jarman, M., and Brodie, A. M. H. 4-Hydroxy-androstenedione treatment for postmenopausal patients with advanced breast cancer. Steroids, *50:* 245–252, 1987.
16. Brodie, A. M. H., Coombes, R. C., and Dowsett, M. Aromatase inhibitors: basic and clinical studies. J. Steroid Biochem., *27:* 899–903, 1987.
17. Schieweck, K., Bhatnagar, A. S., and Matter, A. CGS 16949A, a new non-steroidal aromatase inhibitor: effects on hormone-dependent and -independent tumors *in vivo*. Cancer Res., *48:* 834–838, 1988.
18. Steele, R. E., Mellor, L. B., Sawyer, W. K., Wasvary, J. M., and Browne, L. J. *In vitro* and *in vivo* studies demonstrating potent and selective estrogen inhibition with the non-steroidal aromatase inhibitor CGS 16949A. Steroids, *50:* 147–161, 1987.
19. Santen, R. J., Demers, L. M., Adlercreutz, H., Harvey, H., Santner, S., Sanders, S., and Lipton, A. Inhibition of aromatase with CGS 16949A in postmenopausal women. J. Clin. Endocrinol. Metab., *68:* 99–106, 1989.
20. Lipton, A., Harvey, H. A., Demers, L. M., Hanagan, J. R., Mulagha, M. T., Kochak, G. M., Fitzsimmons, S., Sanders, S. J., and Santen, R. J. A phase 1 trial of CGS 16949A. Cancer (Phila.), *65:* 1279–1285, 1990.
21. Vanden Bossche, H., Willemsens, G., Roels, I., Bellens, D., Moereels, P., Coene, M. C., Le Jeune, L., Lauwers, W., and Janssen, P. A. J. R76713 and enantiomers: selective, non-steroidal inhibitors of the P450-dependent oestrogen synthesis. Biochem. Pharmacol., *40:* 1707–1718, 1990.
22. Wouters, W., De Coster, R., Krekels, M., van Dun, J., Beerens, D., Haelterman, C., Raeymakers, A., Freyne, E., van Gelder, J., Venet, M., and Janssen, P. A. J. R76713, a new non-steroidal aromatase inhibitor. J. Steroid Biochem., *32:* 781–788, 1989.
23. De Coster, R., Wouters, W., Browden, C. R., VandenBossche, J., Bruynseels, J., Tuman, R. W., Van Ginckel, R., Snoeck, E., Van Peer, A., and Janssen, P. A. J. New non-steroidal aromatase inhibitors: focus on R76713. J. Steroid Biochem. Mol. Biol., *37:* 335–341, 1990.
24. Tuman, R. W., Morris, D. M., Wallace, M. M., and Bowden, C. R. Inhibition of peripheral aromatization in the cynomolgus monkey by a novel non-steroidal aromatase inhibitor (R76713). J. Clin. Endocrinol. Metab., *72:* 755–760, 1991.
25. Wouters, W., De Coster, R., Beerens, D., Doolaege, R., Gruwez, J. A., Van Camp, K., Van der Pas, H., and Van Herendael, B. Potency and selectivity of the aromatase inhibitor R76713. A study in human ovarian, adipose stromal, testicular and adrenal cells. J. Steroid Biochem., *36:* 57–65, 1990.
26. De Coster, R., Van Ginckel, R. F., Callens, M. J. L., Goeminne, N. K. G., and Janssens, B. L. E. Antitumoral and endocrine effects of (+)-vorozole in rats bearing dimethylbenzanthracene-induced mammary tumors. Cancer Res., *52:* 1240–1244, 1992.
27. Krekels, M. D. W. G., Wouters, W., Van Ginckel, R., Janssens, B., Callens, M., and De Coster, R. Aromatase inhibition by R83842, the dextro isomer of R76713, in JEG-3 choriocarcinoma grown in ovariectomized nude mice. J. Steroid Biochem. Mol. Biol., *41:* 761–764, 1992.
28. Wouters, W., De Coster, R., Van Dun, J., Krekels, M. D. W. G., Dillen, A., Raeymakers, F., Freyne, E., Van Gelder, I., Sanz, G., Venet, M., and Janssen, M. Comparative effects of the aromatase inhibitor R76713 and its enantiomers on steroid biosynthesis in rat cells *in vitro* and *in vivo*. J. Steroid Biochem. Mol. Biol., *36:* 1049–1054, 1990.
29. De Coster, R., Bruynseels, J., Van Ginckel, R., Janssens, D., Vanherck, W., Goeminne, N., and Doolaege, R. Endocrine and antitumoral effects of R76713, a new non-steroidal aromatase inhibitor. J. Steroid Biochem., *36* (Suppl.)*:* 119S, 1990.
30. Poortman, J., Thijssen, J. H. H., and Schwarz, F. Androgen production and conversion to estrogens in normal postmenopausal women and in selected breast cancer patients. J. Clin. Endocrinol. Metab., *37:* 101–109, 1973.
31. Thijssen, J. H. H., Blankenstein, M. A., Donker, G. H., and Daroszewski, J. Endogenous steroid hormones and local aromatase activity in the breast. J. Steroid Biochem. Mol. Biol., *39:* 799–804, 1991.
32. Thijssen, J. H. H., Wood, W. G., and Kessler, A. C. Multicentre evaluation of enzyme-linked immunoassays of follitropin and lutropin in serum or plasma. Clin. Chem., *37:* 1257–1263, 1991.
33. Van Landeghem, A. A. J., Poortman, J., Thijssen, J. H. H., Deshpande, N., Di Martino, L., Tarquini, A., and Schwarz, F. Plasma concentration gradient of steroid hormones across human mammary tumours *in vivo*. J. Steroid Biochem., *14:* 741–747, 1981.