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Advances in Brief

Inhibition of Breast Cancer Tissue Aromatase Activity and Estrogen Concentrations by the Third-Generation Aromatase Inhibitor Vorozole

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Abstract

In about one-third of advanced breast cancers, estrogen deprivation causes tumor regression. Estrogen concentrations in tumor tissue seem to depend largely on local production. The aromatase enzyme complex is thought to be the key enzyme in this respect. In the present study, the effect of the new third-generation nonsteroidal aromatase inhibitor vorozole (Rivizor) on tumor tissue aromatase activity and estrogen concentrations was evaluated. During 7 days preceding mastectomy, 11 postmenopausal breast cancer patients were treated with 2.5 mg of vorozole once daily. Eight patients could be evaluated. Intratumoral aromatase activity and estrone and estradiol levels were measured and compared to the values of nine untreated postmenopausal breast cancer patients. In treated patients, median tissue aromatase activity was 89% lower than that in controls ($P < 0.001$). Similarly, median tissue estrone and estradiol concentrations were 64 and 80% lower, respectively, in treated patients ($P = 0.001$ and $P < 0.05$, respectively). These results support the hypothesis that depleting the tumor of estrogens, thus impairing estrogenic stimulation, is an important mechanism in the antitumor activity of aromatase inhibitors.

Introduction

In about one-third of advanced breast cancers, estrogens stimulate tumor growth, whereas estrogen deprivation causes tumor regression (1). Inhibition of estrogenic stimulation is the main target in endocrine treatment of breast cancer. This is achieved either by blocking the estrogen receptor or by the inhibition of estrogen production. The cytochrome P-450 enzyme complex aromatase is the main target for inhibition of estrogen production. Aromatase catalyzes the conversion of the androgens androstenedione and testosterone to estrone and estradiol, respectively. In recent years, several new aromatase inhibitors have been developed. One of these is vorozole (Rivizor, R83842), a triazole derivative, which is a nonsteroidal, potent, and highly specific aromatase inhibitor (2–4). Vorozole has been developed from the racemic mixture R76713. The aromatase activity of R76713 has been shown to reside almost exclusively within the dextro-isomer vorozole. In healthy postmenopausal women, a single oral dose of 1–5 mg of the racemate resulted in an almost complete inhibition of peripheral aromatase activity (5). In Phase II clinical investigations in postmenopausal patients with metastatic breast cancer, 2.5 mg of vorozole once daily, as a second-line treatment after tamoxifen, was shown to be an effective treatment without any significant clinical side effects (6). One Phase III study showed equivalent efficacy of vorozole (2.5 mg once daily) to aminoglutethimide (250 mg twice daily) plus hydrocortisone (30 mg/day) as a second-line treatment after tamoxifen in postmenopausal patients with metastatic breast cancer (7).

After menopause, serum estrogen levels decrease considerably. In breast cancer tissue, however, pre- and postmenopausal estrogen levels do not differ significantly (8). Therefore, in postmenopausal patients, a serum to tumor gradient exists. There is evidence that intratumoral estrogen production is mainly responsible for high tumor estrogen levels (9, 10). The aromatase enzyme complex is thought to have an important role in this respect (11). Tumor aromatase activity was found to be a good predictor of response to treatment with the aromatase inhibitor aminoglutethimide (12). Changes in tumor aromatase activity after treatment with two aromatase inhibitors, aminoglutethimide and 4-hydroxyandrostenedione, have been reported (12, 13). For patients treated with 4-hydroxyandrostenedione, a decrease in aromatase activity in most tumors was found, whereas treatment with aminoglutethimide caused a paradoxical rise in aromatase activity. Suggested reasons for this paradoxical rise were the induction of aromatase by hydrocortisone comedication and the induction of aromatase and other microsomal enzymes by aminoglutethimide. Thus tumor aromatase activity is influenced differently after the administration of different aromatase inhibitors. To date, there have been no reports on the effects of aromatase inhibitors on tissue estrogen levels. The effect of the administration of vorozole on tumor aromatase activity is unknown, as is its effect on intratumoral estrogen levels. The present study was designed to evaluate these effects.

Materials and Methods

Eleven women were included in the study. The inclusion criteria were: (*a*) postmenopausal status; (*b*) scheduled surgery for suspected breast cancer larger than 2 cm in diameter; and (*c*) informed consent. Exclusion criteria were: (*a*) premenopausal status; (*b*) prior treatment with endocrine or chemotherapy; (*c*) intestinal disorders leading to possible abnormal resorption; (*e*) endocrine disorders (not including well-regulated diabetes mellitus); (*f*) abnormal liver function tests; (*g*) abnormal renal function tests; and (*h*) the use of etomidate in anesthesia.

During the 7 days preceding surgery, the patients ingested the investigational drug in a single dose between 8 and 9 a.m., before breakfast. The first four patients were treated with 5 mg of the racemate R76713; the remainder received vorozole (R83842) at the equivalent dose of 2.5 mg. The last tablet was taken on the morning of the day before the operation. During surgery, as much tumor tissue as possible was collected and immediately frozen at −80°C. In this tissue, estrogen and progesterone receptor levels, aromatase activity, and estrone and estradiol levels were measured. Estrogen and progesterone receptor levels were measured quantitatively using the ligand binding assay according to the guidelines of the European Organization for Research and Treatment of Cancer (EORTC) Receptor Study Group. Tissue estrogen levels and aromatase activity were measured using procedures described previously (11, 14). Results are expressed as femtomoles of estradiol converted/milligram of protein/2 h. Protein was measured according to Bradford (15) using human serum albumin as a standard. As controls, nine samples of breast cancer tissue

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of operated postmenopausal patients, matched for age and receptor status, were taken from our tumor bank. This tissue was processed identically to that of treated patients.

Results

Patient characteristics are shown in Table 1. No adverse events were reported during treatment with either vorozole or R76713. The average age of treated patients and controls did not differ significantly (70 and 67 years, respectively; $P = 0.54$). Estrogen and progesterone receptor levels were predominantly positive in patients and controls. Tissue samples from 8 of 11 patients were available for analysis. Three patients were finally excluded from analysis because of progressive disease limiting drug intake, possible premenopausal status, and benign tumor histology at pathological examination (patients T1, T3, and T4, respectively; Table 1).

Tumor aromatase activity was measured in all patients treated with vorozole. Depending on the remaining amount of tissue, measurement of estrogen and progesterone receptor levels and tissue estrone and estradiol levels was performed. As shown in Fig. 1, aromatase activity in tumor tissue from treated patients ($n = 8$; range, 0.27–6.60 fmol/mg protein/2 h; median, 0.80; mean, 1.46; SD, 2.12) was significantly lower than that in tumor tissue from control patients ($n = 9$; range, 2.40–18.81 fmol/mg protein/2 h; median, 7.19; mean, 7.95; SD, 4.96; $P < 0.001$) using a two-sided Mann-Whitney test. Median tissue aromatase activity in tumors from treated patients was 89% lower than that in controls. The amount of tissue was sufficient to measure tissue estrogen levels in only five patients. Although the number of measurements is smaller, the endogenous estrogen levels were also significantly lower than those in control patients [estrone: patients ($n = 5$; range, 160–335 fmol/g tissue; median, 280; mean, 273; SD, 67) *versus* controls ($n = 9$; range, 575-1630 fmol/g tissue; median, 780; mean, 951; SD, 391), $P = 0.001$; estradiol: patients ($n = 5$; range, 110–250 fmol/g tissue; median, 170; mean, 168; SD, 52) *versus* controls ($n = 9$; range, 148-1770 fmol/g tissue; median, 850; mean, 840; SD, 575), $P < 0.05$]. Median tissue levels of estrone and estradiol were 64 and 80% lower than those in control tissues, respectively. Estrogen concentrations in control tissues were in good agreement with our previous observations (10).

Table 1 *Age, menopausal status, estrogen receptor (ER) and progesterone receptor (PgR) concentrations of treated patients (T) and controls (C)*

	Age (yrs)	Menopausal status[a]	ER (fmol/mg protein)	PgR (fmol/mg protein)
T1	75	Post	35	379
T2	52	Post	0	42
T3	45	Pre	44	660
T4	46	Post	1	0
T5	47	Post	65	65
T6	88	Post	Unknown	Unknown
T7	62	Post	Unknown	Unknown
T8	78	Post	181	0
T9	74	Post	Unknown	Unknown
T10	79	Post	127	0
T11	78	Post	127	0
C1	53	Post	8	0
C2	55	Post	17	0
C3	62	Post	54	0
C4	85	Post	81	629
C5	68	Post	649	246
C6	73	Post	79	226
C7	76	Post	56	212
C8	64	Post	42	394
C9	70	Post	0	0

[a] Post, postmenopausal; pre, premenopausal.

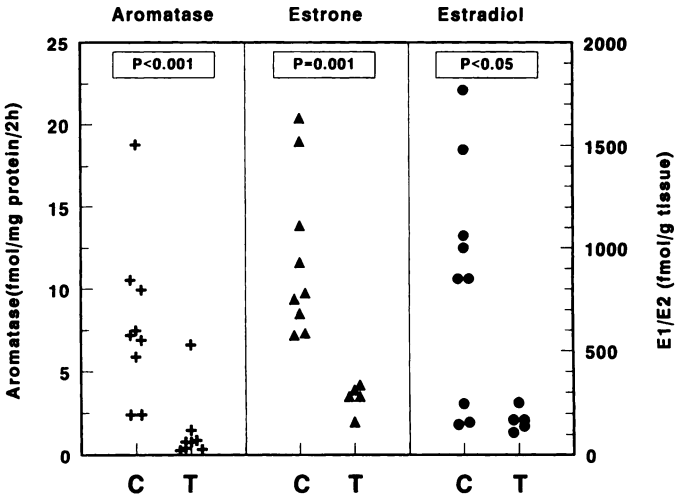

Fig. 1. Aromatase activity and estrogen levels in breast cancer tissue from untreated patients (*C*) and from patients treated with 2.5 mg of vorozole daily for 7 days (*T*). Median aromatase activity, estrone concentrations, and estradiol concentrations were 89, 64, and 80% lower, respectively, in patients treated with vorozole.

Discussion

The results of this study confirm our hypothesis that vorozole is able to significantly inhibit tumor aromatase activity in breast cancer tissue. Thus, vorozole is not only able to inhibit peripheral aromatase activity (5) but also inhibits tumor aromatase activity. In addition, the present study is the first to report that treatment with vorozole causes a decrease in endogenous estrogen concentrations in breast tumor tissue.

To our knowledge, vorozole is the first aromatase inhibitor for which this phenomenon has been found. These results support the hypothesis that depleting the tumor of estrogens and thus impairing estrogenic stimulation is an important mechanism in the antitumor activity of aromatase inhibitors.

Decreased *in vitro* aromatase activity could, in theory at least, be attributed to residual vorozole in the tissue sample. The techniques used do not allow for washing away such residues, should they exist. This possibility is considered highly unlikely, however, in view of the observed decrease in the concentration of estrogens (Fig. 1).

Our findings are in concordance with the results of Reed *et al.* (13), who found that 4-hydroxyandrostenedione is able to inhibit tumor aromatase activity. Miller and O'Neill (12), by contrast, found that tumor aromatase activity increased after treatment with aminoglutethimide. These authors suggested that a general induction of cytochrome P-450 enzymes and the concurrent use of hydrocortisone could be responsible for this increase. Enzyme induction could be a factor in the development of tumor resistance to treatment with aminoglutethimide. In terms of enzyme induction, short-term treatment with vorozole seems to resemble 4-hydroxyandrostenedione rather than aminoglutethimide. The present study does not allow a definite conclusion in this respect. Whether the specificity of vorozole for the aromatase enzyme complex and/or the duration of treatment contribute to its effect on the aromatase enzyme complex has yet to be tested in long-term clinical investigation.

Reed *et al.* (13) and Miller and O'Neill (12) compared aromatase activity in breast tumor tissue before and after treatment with an aromatase inhibitor in the same patients. In our study, it was not possible to obtain tumor tissue specimens twice in the same patient due to ethical considerations, although this would have been the preferred method for best comparison. Therefore, aromatase activity

and estrogen concentrations in treated and control patients were compared.

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