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Clinical paper

Vorozole (R83842) in the treatment of postmenopausal advanced breast cancer: relationship of serum levels of vorozole and clinical results (a study of the EORTC Breast Cancer Cooperative Group)

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The new non-steroidal aromatase inhibitor vorozole (R83842) was administered orally at a single daily dose of 2.5 mg to 27 postmenopausal women with advanced breast cancer in a phase II trial as second-line endocrine treatment. The observed objective response rate of 30% and good tolerability of the drug confirm other recent reports. Endocrine determinations during 6 months of treatment demonstrated reduction of serum estrogens: estrone sulfate by more than 60%, estrone by 30–40%, but estradiol by only 10–20%. The ratios of serum androstenedione/estrone and testosterone/estradiol increased significantly, consistent with the inhibition of peripheral aromatase activity. Levels of progesterone, 17-α-hydroxyprogesterone, cortisol, dehydroepiandrosterone sulfate, androstenedione and aldosterone remained normal, indicating no interference with adrenocortical steroid synthesis. A general lack of correlation between the observed serum concentrations of vorozole and its effect on hormone serum levels or clinical response was found. This suggests that the determination of such serum levels gives a poor impression of the unambiguous anti-tumor activity of vorozole which may well have its main effect with the tumor tissue itself. The present results are in support of aromatase inhibition, but the possibility of an additional effect on the sulfation of estrogens merits further investigation. [© 1998 Lippincott-Raven Publishers.]

Key words: Breast cancer, EORTC, R83842, vorozole.

Introduction

Vorozole (R83842) is the active dextro-enantiomer of the racemic compound R76713, which is a new non-steroidal potent aromatase inhibitor.[1] Recent clinical phase I trial experience with R76713 in postmenopausal patients with pretreated advanced breast cancer has demonstrated that the drug effectively suppresses serum levels of estradiol and estrone within 2 weeks of treatment at a daily dosage of 2.5 or 5 mg.[2] In the 16 patients studied in this trial, serum estrogens dropped without a noticeable change of serum cortisol, aldosterone or adrenocortical reponsiveness to synthetic ACTH.[2] Tracer studies *in vivo* showed that racemic R76713 caused 90-95% inhibition of the conversion of androstenedione to estrone.[3] In a randomized phase II clinical trial, daily doses of 1, 2.5 or 5 mg of R83842 given to postmenopausal patients with advanced breast cancer progressing on treatment with tamoxifen, reduced serum estradiol levels by 90%.[4] A phase II clinical trial of R83842 2.5 mg daily in patients with tumor progression on tamoxifen treatment demonstrated a similar selective suppression of serum estrogens.[5] In the latter study three out of 29 patients showed a partial response with a median duration of 15 months, whereas stable disease lasting a median period of 12 months was observed in 14 patients. Tolerability of the drug was reported as excellent.

In December 1991 the EORTC Investigational Drug Branch for Breast Cancer (IDBBC) initiated a phase II clinical trial of vorozole as second-line endocrine therapy in postmenopausal women with advanced breast cancer. The clinical results, which confirmed

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the previously reported anti-tumor activity of vorozole,[4,5] have been published separately.[6] Here, we report on the serum levels of the drug and several endocrine parameters of interest in 16 of the 27 participating patients during the first 6 months of treatment. We could only demonstrate a generally very weak or absent correlation between drug and hormone levels in serum, and the absence of a correlation between such levels and the clinical effects. The usefulness of the measurement of serum levels in patients treated with an aromatase inhibitor like vorozole will be discussed.

Patients and methods

Patients

Twenty-seven patients were entered in the study between December 1991 and February 1993. Six centers from Belgium, the Netherlands and the UK participated in the trial. The study was approved by the Protocol Review Committee of the EORTC and the ethics committee of each participating center. To be eligible for the study, patients had to have histologically or cytologically verified breast cancer with uni- or bi-dimensionally measurable metastatic lesions. Tumors had to be receptor positive (estrogen receptor and/or progesterone receptor levels higher than 10 fmol/mg protein as determined by the dextran charcoal coated method) or receptor status unknown, but in the latter case the relapse-free survival had to be more than 1 year. A postmenopausal status was mandatory and defined as the absence of regular menses for a minimum of 12 months. Follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol levels were to be determined for women below the age of 56 who had undergone either hysterectomy before the onset of menopause, or bilateral oophorectomy or radiocastration, or who presented amenorrhea after stopping oral contraceptives. In such cases, increased FSH and LH in the presence of postmenopausal plasma estradiol concentrations had to be documented. A patient performance status below 3 and a life expectancy of at least 3 months were required. Patients could have received prior chemotherapy or hormonotherapy as adjuvant treatment. A maximum of one previous hormonal therapy for metastatic disease was allowed as long as 2 weeks had elapsed between the discontinuation of this treatment and the start of therapy with vorozole. If the most recent endocrine therapy was ovarian ablation, an interval of at least 3 months since the day of oophorectomy was required. A written informed consent was obtained from all patients. Patients with central nervous system metastases were excluded as well as patients with rapidly progressive life-threatening metastases.

The assessment of tumor parameters was done every 2 months. Vorozole was given orally, at the dose of 2.5 mg once daily to be taken in the morning until disease progression or excessive toxicity.

A more elaborate description of the clinical study protocol has been reported elsewhere.[6]

Serum assays

Non-fasting 30 ml blood samples were drawn on an outpatient basis in the morning or afternoon before treatment, at 2 weeks, and 1, 2, 4 and 6 months of treatment (depending on the duration of treatment with vorozole). Blood samples were centrifuged for 10 min at 1000 *g* within 2 h after collection. Serum aliquots were stored in plastic tubes at −20°C until analysis.

The determination of vorozole concentrations was done by the Janssen Research Foundation (Dr R Woestenborghs, Beerse, Belgium) by gas chromatography with electron-capture detection (limit of quantification 1.0 ng/ml) or high-pressure liquid chromatography (HPLC) with UV detection (limit of quantification 5.0 ng/ml). All hormonal assays were performed at the Netherlands Cancer Institute in Amsterdam.

Estradiol (E_2), testosterone (T) and progesterone (P) were determined by the Spectria coated tube radioimmunoassay (RIA) from Orion Diagnostica (Espoo, Finland).

Sex hormone binding globulin (SHBG) was measured by the Spectria IRMA method from the same manufacturer.

Estrone (E_1) was measured by RIA (Diagnostic Systems, Webster, TX); estrone sulfate (E_1S) was determined according to Samojlik *et al.*[7] Cortisol (F), aldosterone (Aldo) and 17-α-hydroxyprogesterone (17-OH-P) were assayed by coat-A-count RIA (Diagnostic Products, Los Angeles, CA). Dehydroepiandrosterone sulfate (DHEA-S) and Δ-4-androstenedione (A) were determined as previously published.[8] For the measurement of A a slight modification was introduced: after hexane:ethylacetate 98:2 v/v extraction of the NaOH-treated serum, drying and redissolution in 0.01 M phosphate buffer pH 7.4 containing 0.25% BSA, incubation followed with 1:21 000 diluted rabbit antiserum raised against A-6-hemisuccinate-BSA and 1,2-[^{3}H]A used as a tracer at 4°C during 18 h. Separation of bound and unbound A was performed with dextran-coated charcoal.

Performance data for these assays are presented in Table 1.

Due to insufficient serum material the number of determinations per time point per assay were not always the same.

Statistics

For comparison between individual serum levels at different points in time the Mann-Whitney-Wilcoxon rank sum test was applied. The Spearman coefficient was calculated to determine the correlation between serum concentrations of vorozole and endocrine parameters. The clinical response to treatment [complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) according to UICC criteria] was compared to serum levels of both vorozole and hormonal parameters with both the Spearman test and the one-way analysis of variance.

Results

Clinical

The age of the 27 patients evaluable for toxicity and tumor response ranged from 39 to 86 years (median 67 years). In 22 patients at least the estrogen receptor (ER) in the primary tumor was known to be positive, in five patients the ER status was unknown. Three patients had been using tamoxifen as adjuvant therapy; 25 patients had used tamoxifen prior to vorozole for metastatic disease, one of these received tamoxifen for both indications.

A complete response was observed in two of the patients, a partial response in six, resulting in an objective response rate of 30%. Stable disease as best response was observed in nine patients, ongoing tumor progression in 10, of whom three showed 'early progression', i.e. within the first 2 months of treatment. The median time to tumor progression was 14.3 (6.8-40.6) months. The clinical patient characteristics are summarized in Table 2. A more detailed report of the treatment results in terms of tumor response and tolerability of the treatment has been published separately.[6]

Hormonal

Table 3 gives the mean serum levels of the endocrine parameters measured during the first 6 months of treatment. A large variation was observed for all hormones measured. The average level of A hardly changed. Figure 1 demonstrates how the mean serum levels of E_1 and E_1S decreased to 50-75% of their initial value within 2 weeks, the effect lasting during the 6 months observation period. Figure 2 shows that the individual A/E_1 ratio rose to twice the initial value. The serum levels of E_2 showed a relatively large variation, but on average remained within the

Table 1. Performance data of the endocrine assays used.

Assay	Sensitivity	Intra CV[b] (%)	Inter CV[b] (%)
Estrone	5 pmol/l	5.6	11.1
Estrone sulfate	0.05 nmol/l	11	15
Estradiol	20 pmol/l	9.7	5.1
Androstene-dione	0.3 nmol/l	7.5	11
Testosterone	0.1 nmol/l	7.5	7.0
Cortisol	0.005 μmol/l	3	6.3
DHEA-S	0.10 μmol/l	6	10
Progesterone	0.3 nmol/l	7.9	8.1
17-OH-P	0.21 nmol/l	5.6	4.8
Aldosterone	44 pmol/l	8.7	10.4
SHBG	0.5 nmol/l	5.5	6.9
TSH	0.1 mIU/l	4.7	9.3
FSH	0.2 IU/l	4.7	11

[a]Limit of quantification.
[b]Coefficient of variation.

Table 2. Patient characteristics ($n = 27$)

Median age [years (range)]	67 (39-86)
Median interval till first recurrence of disease [years (range)]	4.2 (0-19.0)
ER positive/PgR positive or unknown	18
ER positive/PgR negative	4
ER unknown/PgR unknown	5
Prior adjuvant therapy with tamoxifen	2
chemotherapy	5
both	1
oophorectomy	1
Prior tamoxifen for advanced disease	25
Best response to tamoxifen CR	5
PR	5
NC	11
progression	1
not evaluable	3
Median duration of response to tamoxifen [months (range)]	25 (10-43)
Dominant site of disease	
soft tissue	4
bone	11
visceral	12

Table 3. Serum levels of hormones and vorozole during 6 months of treatment (mean ± SD) (the number of samples measured is indicated between parentheses; individual values before treatment and at later points in time were compared with the Mann–Whitney Wilcoxon rank sum test)

	Before treatment	2 weeks	1 month	2 months	4 months	6 months
Vorozole R83482 (μg/l)		54.4 ± 35.2 (9)	61.7 ± 44.8 (14)	90.3 ± 97.6 (11)	40.2 ± 29.3 (10)	36.6 ± 25.1 (9)
Estrone (pmol/l)	107 ± 53 (14)	66 ± 49 (11)[a]	83 ± 81 (13)	66 ± 31 (11)[a]	73 ± 34 (11)	48 ± 19 (9)[b]
Estrone sulfate (nmol/l)	0.92 ± 0.27 (12)	0.40 ± 0.09 (11)[c]	0.51 ± 0.26 (13)[b]	0.43 ± 0.13 (10)[c]	0.58 ± 0.32 (10)[a]	0.40 ± 0.15 (8)[c]
Estradiol (pmol/l)	52 ± 22 (15)	41 ± 20 (11)	44 ± 26 (15)	45 ± 22 (12)	55 ± 27 (11)	41 ± 21 (9)
Androstenedione (nmol/l)	1.67 ± 0.74 (13)	1.77 ± 0.62 (11)	1.64 ± 63 (15)	1.92 ± 0.67 (11)	2.06 ± 1.02 (10)	1.85 ± 1.04 (8)
Testosterone (nmol/l)	1.02 ± 0.53 (16)	1.20 ± 0.67 (11)	1.12 ± 0.65 (15)	1.32 ± 0.79 (12)	1.28 ± 0.79 (11)	0.89 ± 0.19 (9)
Cortisol (μmol/l)	0.47 ± 0.20 (16)	0.50 ± 0.21 (11)	0.42 ± 0.17 (15)	0.42 ± 0.20 (12)	0.37 ± 0.22 (11)	0.40 ± 0.12 (9)
DHEA-S (μmol/l)	1.25 ± 1.02 (16)	1.18 ± 0.92 (11)	1.31 ± 0.87 (15)	1.09 ± 0.77 (12)	1.26 ± 1.23 (11)	0.87 ± 0.72 (9)
Progesterone (nmol/l)	1.09 ± 0.49 (16)	0.89 ± 0.48 (11)	1.05 ± 0.56 (15)	1.00 ± 0.47 (12)	1.15 ± 0.53 (11)	0.91 ± 0.36 (9)
17 OH-P (nmol/l)	1.5 ± 0.7 (16)	1.5 ± 0.6 (11)	1.6 ± 1.1 (15)	1.7 ± 0.9 (12)	2.3 ± 1.9 (11)	1.7 ± 0.8 (9)
Aldosterone (pmol/l)	522 ± 692 (16)	580 ± 722 (11)	398 ± 259 (15)	479 ± 391 (12)	380 ± 191 (11)	360 ± 225 (9)
Androstenedione /estrone	1.61 ± 0.54 (13)	3.49 ± 1.71 (11)[c]	3.16 ± 2.53 (13)[b]	3.48 ± 2.10 (11)[c]	3.01 ± 1.24 (10)[b]	4.43 ± 4.23 (8)[c]
Testosterone /estradiol	2.02 ± 0.75 (15)	3.25 ± 1.66 (11)[a]	2.87 ± 1.20 (15)[a]	3.02 ± 0.82 (12)[b]	2.52 ± 1.03 (11)	2.58 ± 1.20 (9)
Estrone/estrone sulphate	136 ± 79 (12)	166 ± 129 (11)	182 ± 173 (12)	147 ± 55 (10)	138 ± 63 (10)	130 ± 46 (8)
SHBG (nmol/l)	106.9 ± 59.9 (16)	100.5 ± 46.3 (11)	92.2 ± 52.6 (15)	90.2 ± 41.0 (12)	73.5 ± 46.3 (11)	68.0 ± 32.4 (9)
TSH (mU/l)	5.8 ± 13.1 (13)	1.4 (1)	0.3 (1)	1.4 ± 0.1 (2)	1.3 ± 0.7 (5)	4.3 ± 9.0 (9)

[a] $p \leq 0.05$; [b] $p \leq 0.01$; [c] $p \leq 0.001$.

normal range. Only four out of 73 samples contained less than 20 pmol/l, which was the lower detection limit of the assay. The E_1/E_1S ratio showed a tendency to increase during the first month of treatment (statistically not significant), returning to base-line values thereafter.

The serum levels of cortisol and its precursor 17-α-OH-P remained stable. P and DHEA-S levels did not change either, and also aldosterone levels remained unaffected. The serum concentration of SHBG, regarded as the main determinant of the bioavailability of E_2 and T, gradually fell during treatment, although this phenomenon was not statistically significant.

Thyrotropin (TSH) concentrations, included to check for thyroidal dysfunction, did not rise. Two patients had abnormally high values at the start of treatment, one of which had to be subsequently treated by replacement therapy.

Vorozole levels versus hormonal and clinical results

The median serum concentration of vorozole was 49.0 ng/ml (mean ± SD; 52.3 ± 42.9 ng/ml). We have tested the Spearman correlation between serum concentrations of vorozole and the different hormone levels, expressed as a percentage of the serum concentration just prior to therapy with vorozole in 40-53 samples. The best correlation was observed with E_1S ($r = -0.40$; $p = 0.01$) and E_1/E_1S ($r = 0.39$; $p = 0.01$). Weak positive correlations were found between vorozole and T ($r = 0.26$; $p = 0.04$) and A ($r = 0.28$; $p = 0.04$). A weak and marginally significant correlation was observed between vorozole levels and A ($r = 0.31$; $p = 0.05$). No correlation was observed for the other hormones measured, notably E_1, E_2 or the ratios T/E_2 or A/E_1.

When tumor response categories (i.e. objective tumor regression, SD or PD) were compared with drug or hormone serum levels (the latter as a percentage of the value before treatment) at 8 weeks of treatment, no correlation was found with the exception of a strong positive correlation between lack of tumor response (i.e. tumor progression) and 17-α-OH-P. At 16 weeks, when patients with PD had been switched to other treatment modalities, no such correlation was seen.

The clinical toxicity varied, but in general toxicity was so low that no correlation could be observed with vorozole serum levels.

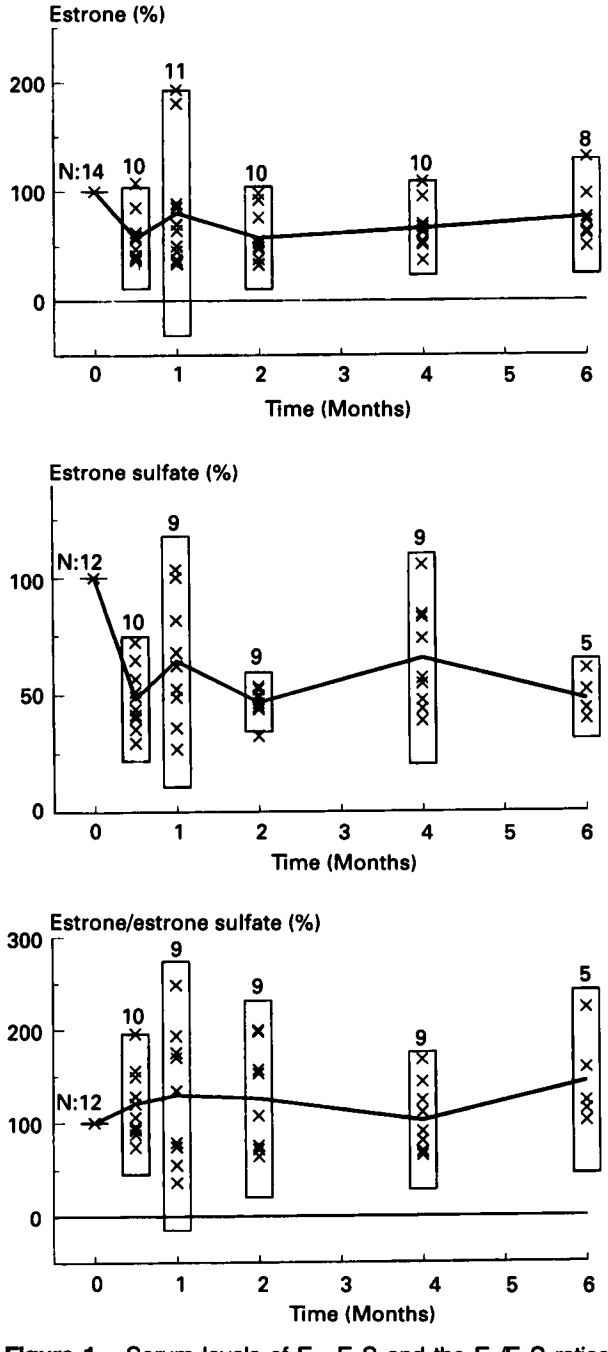

Figure 1. Serum levels of E_1, E_1S and the E_1/E_1S ratios (mean ± 2 SD) before and during 6 months of treatment with vorozole.

Discussion

This study of hormone levels and drug concentrations in the serum of postmenopausal patients treated with vorozole for metastatic breast cancer has shown that the drug reduces serum E_1 and its sulfate to some extent, but that E_2 levels are hardly affected. The reduction of E_1 and E_1S observed in the present study is very similar to data reported recently by others.[4,5] The reduction of serum E_2 in our patients, however, was much smaller than what was described. The small decrease of E_2 cannot be explained by an assay technique that was not sensitive enough to detect relatively low serum concentrations, since the method used is suitable for the reproducible measurement of concentrations as low as 20 pmol/l and only four samples contained E_2 concentrations below 20 pmol/l. The decrease of E_1 and E_1S is most likely the result of an inhibition of conversion of A by aromatase, which has been reported before.[3] The rise of the individual A/E_1 and T/E_2 ratios is in agreement with this. The rise of the A/E_1 ratio seems more than might be expected from the average decrease of E_1 levels to approximately 70%, as shown in Figure 1. As with the aromatization of A to E_1, the individual T/E_2 ratio seemed a more sensitive indicator of the inhibition of the conversion of T to E_2 (Table 3). E_1S was decreased to a greater extent than E_1, suggesting that vorozole

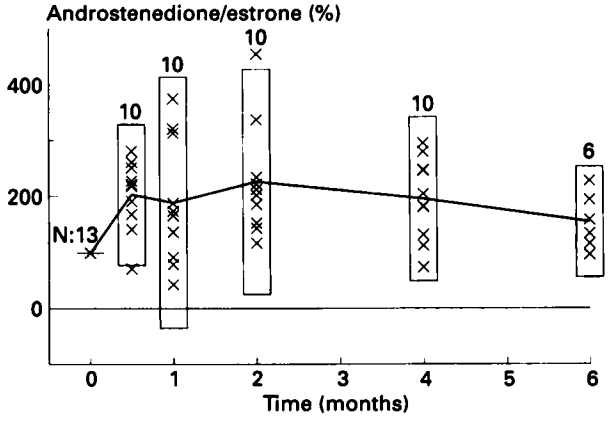

Figure 2. Serum A/E_1 ratios (mean ± 2 SD) before and during 6 months of treatment with vorozole.

may also have an effect on the metabolism of E_1S. The significance of such an effect for the therapeutic results remains speculative. The therapeutic meaning of the modest decrease of serum estrogen levels which, except for E_1S, had no relation with the vorozole concentrations measured at the same points in time is unclear. Although the reduction of serum estrogens in combination with rising A/E_1 and T/E_2 ratios reflects the effects of aromatase inhibition, circulating estrogens are maintained in relatively large amounts. The latter do not substantially differ from what is known for patients treated with low doses of the first generation aromatase inhibitor aminoglutethimide.[9] The likelihood that the reduction of serum estrogens substantially contributes to the therapeutic effect becomes even smaller when we take the decrease of SHBG levels into consideration. Lower SHBG serum concentrations would imply greater availability of estrogen for biological activity, so that the effect of the decrease of serum estrogen levels seems at least partially neutralized by this reduction of SHBG. Tamoxifen is known to increase serum concentrations of SHBG.[10] As has been proposed by Johnston *et al.*,[4] it is probable that the main reason for the observed reduction of SHBG is the cessation of previous treatment with tamoxifen occurring in almost all patients and that the reduction in serum estrogens, which is small in its absolute size, has contributed little in this regard. In our patients tamoxifen treatment had generally been withheld since 4 weeks prior to the start of therapy with vorozole, when tamoxifen clearance may not yet have been complete. However, the decrease of the mean SHBG serum levels seems to be progressive during the 6 months of observation, although this is not statistically significant.

Vorozole concentrations showed little or no correlation with hormonal concentrations, with the exception of E_1S, or the E_1/E_1S ratio and the serum concentration of A. It is understood that the fluctuating nature of serum concentrations of steroids (with the exception of their sulfated derivatives) and the relatively short $T_{1\backslash 2}$ of vorozole (approximately 13 h[11]) contribute to this general lack of correlation. The positive correlation between vorozole levels and the E_1/E_1S ratio indicates that E_1 and E_1S are not equally reduced, therefore suggesting the inhibition of the formation, or the stimulation of the dissociation of E_1S by vorozole. Either effect could easily affect E_1 serum levels, as the serum concentrations of E_1S are approximately 10 times higher than those of E_1.

The serum concentrations of vorozole or estrogens at 8 or 16 weeks of treatment had no correlation with the categories of tumor response. Together with the observed modest reduction of serum estrogens this strongly suggests that serum levels of estrogens or vorozole have little to do with the clinical response. Instead, the proven clinical activity of this drug must be largely or completely at the tumor tissue level. One may also conclude that serum hormone concentrations as here described are only weak indicators of the possible mode of its action. Very recently, De Jong *et al.*[13] have indeed demonstrated effective inhibition of aromatase activity by vorozole in surgical breast cancer specimens obtained from postmenopausal patients who had been treated with 2.5 mg vorozole daily during the week preceding surgery. Our finding that a lack of clinical response was positively related to serum levels of 17-α-OH-P seems best explained by the assumption that patients with progressive disease suffer from greater stress than patients showing a favorable response to the treatment.

Our data show that there is no reason to be concerned about non-specific effects causing gross adrenocortical dysfunction. This is in agreement with preclinical data and other recent results in patients.[4,5]

Unlike aminogluthetimide,[12] vorozole does not seem to interfere with thyroid hormone synthesis, as during this trial no decrease of thyroid function was indicated by a rise of TSH.

It is as yet unclear if the activity of vorozole is explained only by aromatase inhibition at the tumor level. The possibility of an effect on the sulfation of estrogens has to be further investigated.

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