

aromatase inhibitor

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Summary

Vorozole (Rivizor™), is a triazole derivative and one of the new, third generation aromatase inhibitors. Vorozole causes reversible inhibition of cytochrome P₄₅₀ aromatase with the majority of the aromatase inhibition activity attributable to the dextro-isomer. *In vitro* the IC₅₀ against human placental aromatase and in cultured rat ovarian granulosa cells is 1.38 and 0.44 nM, respectively. Vorozole is selective and does not effect other cytochrome P450-dependent reactions at concentrations up to at least 500 fold the aromatase inhibiting concentration. *In vitro* vorozole, at concentrations of up to 10 µM, does not exhibit agonistic or antagonistic effects on steroid receptors including the estrogen, progestin, androgen and glucocorticoid receptors. *In vivo* vorozole produces dose-dependent inhibition of aromatase and reduces circulating estrogen levels. Vorozole has been shown to inhibit intratumoral aromatase activity in postmenopausal breast cancer patients pretreated for 7 days prior to undergoing mastectomy. Tissue estrone and estradiol levels were also shown to be decreased by 64% and 80%, respectively. In four phase II clinical trials, vorozole produced response rates of 18–33% corresponding to selective inhibition of estradiol. Vorozole has been examined in large, randomized multi-centre, controlled trials against both megestrol acetate (MA) and aminoglutethimide (AG) plus hydrocortisone. Against MA, response rates were comparable (10.5% vorozole; 7.6% MA) however, a trend towards improvement in median duration of response for vorozole (18.2 versus 12.5 months; *p* = 0.07) was shown. No differences in time to progression or survival were noted. Significant and persistent weight gain associated with MA administration was the most notable difference in tolerability between the two agents. Against AG, vorozole showed a higher response rate (23% versus 18%) however this did not reach statistical significance (*p* = 0.085). No differences in duration of response, time to progression and survival were noted. A significantly better Functional Living Index-Cancer (FLIC) quality of life score was associated with vorozole compared to AG.

Vorozole is a specific, selective and potent aromatase inhibitor and useful for postmenopausal patients with advanced breast cancer.

Pre-clinical pharmacology

Vorozole is a potent, stereospecific inhibitor of the aromatase enzyme with its activity residing almost

exclusively with the dextro [(+)-(S)]-enantiomer [1]. *In vitro*, using granulosa cells, the dextro-isomer can be shown to cause a dose-dependent inhibition of aromatase with an IC₅₀ of 0.44 nM versus IC_{50s} of

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Compound	Relative potency*
Vorozole (dextro)	1029
Vorozole (racemate)	548
Vorozole (levo)	32
4-Hydroxyandrostene-3,17-dione	34
Aminoglutethimide	1

* The IC₅₀ value (1.42 μM) obtained with aminoglutethimide is used as standard. From Vanden Bossche H, et al. *Biochem Pharmacol* 40: 1716, 1990 (with permission)

0.93 and 240 nM for vorozole racemate and the levo-enantiomer respectively; demonstrating a 545-fold difference in potency between the two enantiomers [1]. In human placental microsomes the dextro-enantiomer is 1.9 times as potent as the racemic mixture and 32 times more potent than the levo form [2].

Against human placental aromatase, the IC₅₀ of the vorozole dextro-isomer is 1.38 nM and the relative potency of vorozole compared to aminoglutethimide is 1,029 versus 34 for 4-hydroxyandrostenedione, a second generation steroidal inhibitor [2] (Table 1). Specificity for aromatase is demonstrated in Table 2 which shows that high concentrations of vorozole are required to inhibit other cytochrome P450-dependent reactions for steroid biosynthesis [1-5] (Table 2). *In vitro* vorozole does not inhibit al-

and generation inhibitor which causes substantial inhibition of mineralocorticoid synthesis [6]. Vorozole concentrations up to 10 μM did not alter the specific binding of estrogens, progestins, glucocorticoids and androgens to their respective receptors *in vitro* [7]. This was confirmed *in vivo* for the estrogen receptor by measuring the ability of vorozole at doses up to 33 mg/kg to induce rat uterine ornithine decarboxylase activity and uterine growth in immature rats [5]. There are no effects, either agonistic or antagonistic, against the uterus as measured by these methods.

In PMSG-stimulated rats, vorozole causes a dose-dependent inhibition of estradiol synthesis two hours after exposure to a single oral dose of the drug. At doses of 0.001 mg/kg and higher, plasma estradiol levels were significantly reduced and the lowest dose producing greater than 95% inhibition was 0.1 mg/kg [8]. The calculated ED₅₀-value was 0.0034 mg/kg [1]. Specificity was confirmed *in vivo* by lack of inhibition of corticosterone and aldosterone production by doses up to 20 mg/kg of vorozole in LHRH/ACTH-injected rats. Testosterone levels were significantly decreased (25 and 41% after administration of 10 and 20 mg/kg vorozole, respectively) and was accompanied by an increase in progesterone and 17α-hydroxyprogesterone levels at the 20 mg/kg dose only. This indicated a partial inhibition of the 17α-hydroxylase/17,20 lyase en-

Table 2. Effects of vorozole on the main cytochrome P450-dependent reactions of steroid biosynthesis

Enzyme	Product formed	Tissue	IC ₅₀ (nM)
14-demethylase	cholesterol	rat liver subcellular fractions	> 10,000
7α-hydroxylase	7α-hydroxycholesterol	rat liver microsomes	> 10,000
cholesterol side-chain cleavage	pregnenolone	bovine adrenal cortex mitochondria	> 10,000
17α-hydroxylase/17,20-lyase	DHEA and androstenedione	bovine adrenal cortex microsomes	> 10,000
17,20-lyase	androstenedione	cultured rat and human* testicular cells	≥ 10,000
17,20-lyase	androstenedione, DHEA, testosterone	rat testis subcellular fractions	1,800
21-hydroxylase	11-deoxycortisol and 11-deoxycorticosterone	bovine adrenal microsomes	> 10,000
		cultured rat and human* adrenal cells	> 10,000
11-hydroxylase	cortisol and corticosterone	bovine adrenal cortex mitochondria	> 10,000
	aldosterone	cultured rat and human* adrenal cells	≥ 10,000
		cultured human adrenal cells*	≥ 10,000

* experiments performed with the racemate. (From Vorozole Investigators Brochure 3rd edition, p. 13, May 1995, with permission).

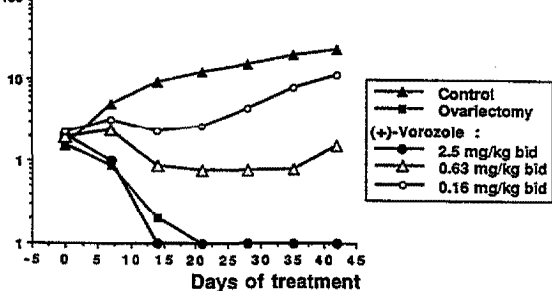


Figure 1. Effect of ovariectomy and treatment with vorozole on the growth of DMBA-induced rat mammary carcinoma. (From Vorozole Investigators Brochure 3rd edition, p. 16, May 1995, with permission)

zyme. In rats fed a sodium-deprived diet for 4 weeks, then given vorozole up to 20 mg/kg daily for 11 days, neither aldosterone, its precursors or plasma renin activity levels were affected [4, 8].

In male cynomolgus monkeys, the effects of vorozole on peripheral aromatization were examined using a double-label, isotope-primed, constant-infusion technique. Conversion of androstenedione to estrone was measured 4–5 hours after dosing. Vorozole caused a dose-dependent inhibition of *in vivo* peripheral aromatization and at an intravenous dose of 130 ng/kg, 50% inhibition of peripheral aromatization was observed [9].

Vorozole was studied using the JEG-3 human choriocarcinoma xenograft in ovariectomized nude mice. The JEG-3 tumor produces estrogens via the aromatization of circulating androgens. In this model vorozole caused a dose-dependent inhibition of intratumoral aromatase activity and a corresponding dose-dependent decrease in uterine weight. Half-maximal reduction in uterine weight and tumor aromatase activity was seen at 0.05 to 0.1 mg/kg of vorozole [8].

Anti-tumor effects

The antitumoral activity of vorozole and vorozole racemate has been evaluated in female Sprague-Dawley rats treated with the carcinogen dimethylbenz(a)anthracene (DMBA) and in female Wistar rats treated with N-methylnitrosourea (NMU) [10,

treatment with 2.5 mg/kg vorozole twice daily for 42 days caused tumor regression comparable to oophorectomy [12] (Figure 1). Using the NMU-induced rat mammary model, treatment with 40 and 160 mg of vorozole racemate/100 grams of food for 42 days reduced tumor growth to the same extent as ovariectomy [5].

Pharmacokinetic profile

After single and multiple dosing, vorozole is shown to have a pharmacological profile in man similar to the rat and dog and different from the rabbit where absorption was slower and there was marked stereoselective metabolism. In dogs, after one month oral dosing, the vorozole tissue to plasma distribution was found to be higher in liver and the adrenal gland [4]. Excretion in the rat and in man is predominantly in the feces and the major metabolite is a desmethyl form [4]. In thirteen postmenopausal breast cancer patients given 2.5 mg vorozole once daily, the T_{max} was approximately 1.1 hours, C_{max} 79.8 ng/ml and the terminal half-life 12.5 hours (steady-state). Steady levels were obtained within 4 days [4]. The AUC and terminal half-life in the postmenopausal breast cancer patients appeared slightly increased compared to pharmacokinetic data from six healthy male volunteers given 2.5 mg vorozole for 2 weeks [C_{max} 63.5 ng/ml; terminal half-life 6.5 hours (steady state)] [4].

Clinical pharmacology

Inhibition of peripheral aromatase activity

A randomized, placebo-controlled, cross-over phase I trial was performed in six healthy male volunteers to study the effect of five different single dose of vorozole (0.25, 0.5, 1, 2.5 and 5 mg) on estradiol levels to identify the lowest effective dose. All doses of vorozole suppressed estradiol levels, however, the lowest dose (0.25 mg) showed a tendency for estradiol levels to escape at 24 hours. No statistical differences between the doses were seen, thus a

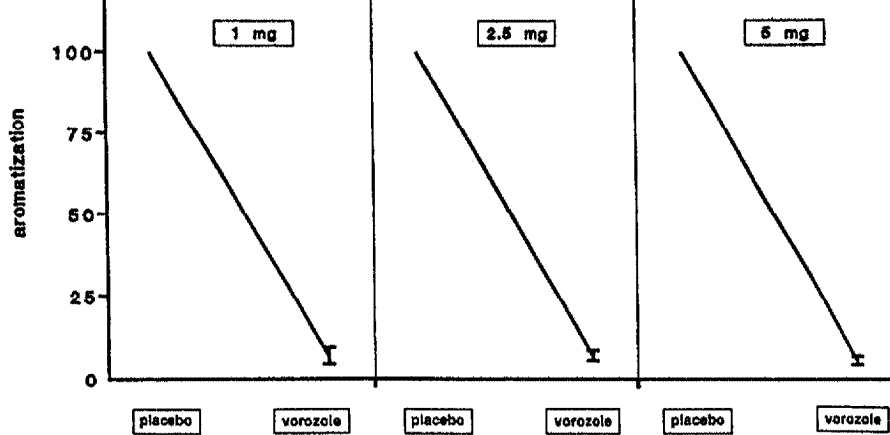


Figure 2. Effect of different doses of vorozole racemate on the *in vivo* peripheral aromatization in normal postmenopausal women. The percentage of conversion during the placebo experiment in the same woman was taken as 100%. After administration of the active compound, the conversion decreased an average of 94%. (From: Van der Wall E, et al., *Cancer Res* 53: 4565, 1993, reprinted with permission.)

minimally effective dose could not be identified. However in the 2.5 and 5 mg groups more estradiol levels were at the assay detection limit [4].

Inhibition of peripheral aromatization was assessed in 12 healthy postmenopausal women who were randomized in a double-blind fashion to receive a single dose of either 1, 2.5 or 5 mg of vorozole racemate. Each woman acted as her own control as an identical experiment was performed with a placebo. Four hours after dosing, [¹⁴C]labeled androstenedione and [³H] labeled estrone were infused and the percentage conversion of androstenedione to estrone assessed. The mean percentage inhibition was 93, 93.2 and 94.4% for 1, 2.5 and 5 mg of vorozole racemate, respectively (Figure 2). No dose response relationship could be established [13].

In a double-blind, placebo-controlled trial, 15 healthy postmenopausal women received 2.5 or 5 mg vorozole racemate once daily for seven days. Median estrone levels were reduced by 71% and 67% for the 2.5 and 5 mg doses, respectively. Median estradiol levels were reduced by 42% in the 5 mg group. No relevant changes in the circulating levels of cortisol, aldosterone, testosterone, progesterone, FSH and LH levels were observed as compared to placebo [4].

In a phase I pilot study 28 postmenopausal breast cancer patients were treated with either 2.5 or 5 mg

of vorozole racemate. These two doses reduced estradiol levels by 45 to 55% and estradiol levels were suppressed to the assay detection limit (10 pmol/l) within 2 weeks and throughout a 4-week period. ACTH stimulation testing was performed in all patients at baseline and after 4 weeks on vorozole and no effect on circulating cortisol or aldosterone was seen at either dose [4].

Intratumoral inhibition

Eleven postmenopausal breast cancer patients were treated with vorozole, 2.5 mg once daily, for seven days preceding mastectomy. Eight patients could be evaluated and intratumoral aromatase activity, estradiol and estrone levels were compared to values from nine untreated postmenopausal breast cancer patients. In the treated group median tissue aromatase activity [median 0.80 fmol/mg protein/2 h (0.27–6.60)] was 89% lower than controls [median 7.19 fmol/mg/protein/2 hr (2.40–18.81)] $p < 0.001$. Additionally, median intratumoral estrone and estradiol levels were also lowered in the treated group by 64 and 80%, respectively. These findings were statistically significant [14].

Treatment Group	VOR-INT-3			VOR-INT-4		
	Vorozole n = 225	Megestrol Acetate n = 227	P Value	Vorozole n = 277	Aminoglutethimide + Hydrocortisone n = 279	P Value
Overall response rate	10.5%*	7.6%*	0.29	23%	18%	0.085
Clinical benefit (CR + PR + NC \geq 6 mths)	–	–	–	47%	37%	0.017
Median duration of response (mths)	18.2	12.5	0.07	20.9	20.4	NS
Median time to progression (mths)	2.7	3.6	0.46	6.7	6.0	NS
Median time to treatment failure (mths)	–	–	–	5.3	4.4	0.040
Median survival (mths)	26.0	28.7	0.93	25.7	21.7	NS
Number AEs leading to withdrawal	7 (3.1%)	14 (6.2%)	–	8 (3%)	29 (10%)	< 0.001

* established by investigator assessment and confirmed by external radiological review.

Choice of vorozole dose for clinical testing

The dose of 2.5 mg of vorozole used in the phase II and III clinical trials was selected based primarily on a phase II, randomized, double-blind, cross-over study where 24 patients received three separate doses of 1.0, 2.5 and 5.0 mg vorozole, each for 4 weeks. Median serum estradiol levels were suppressed by 91, 90, and 89% for the 1, 2.5 and 5 mg doses, respectively. Median estrone levels and estrone sulfate levels were reduced by 54, 55, and 52% and by 69, 64 and 67% for the 1.0, 2.5 and 5 mg doses, respectively. There was a trend ($p = 0.02$) for estradiol to be more frequently suppressed to the assay detection limit [3 pmol/L] with increasing doses of vorozole: 13, 31 and 40% for the 1.0, 2.5 and 5.0 mg doses, respectively. The difference between the 2.5 and 1.0 mg doses (31 versus 13%) was significant in favor of the 2.5 mg dose, however there was no difference between the 2.5 and 5.0 mg doses, thus supporting selection of the 2.5 mg dose for clinical development [15].

Supportive data came from the previously described male volunteer peripheral aromatization study where both the 2.5 and 5 mg groups had a higher proportion of patients having estradiol levels below the assay detection limit than the other doses. However there were no differences between

2.5 and 5 mg of vorozole in the proportion of patients below detection limit values [4].

Clinical studies

Phase II clinical trials

Four phase II clinical trials have been conducted in Canada, the United Kingdom, Europe (EORTC), and Italy [15–19]. In total, 115 patients were enrolled in these studies. Eligible patients were defined as tamoxifen failures – either in the metastatic (had ‘responded’ to previous tamoxifen and subsequently progressed) or adjuvant (relapsed after one year or more of adjuvant tamoxifen) setting. Additionally patients had to have estrogen and/or progesterone receptor positive or unknown disease and have measurable lesions. Responses were evaluated using the International Union Against Cancer (UICC) or European Organisation for Research and Treatment of Cancer (EORTC) criteria. Response rates observed ranged between 18 and 33% [15–19]. The endocrine effects of vorozole in these studies demonstrated a significant reduction in estrone and estradiol. An increase in gonadotrophin levels and a reduction in sex hormone binding globulin (SHBG) were noted in patients who recently

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