Hormonal effects of MPV-2213ad, a new selective aromatase inhibitor, in healthy male subjects. A phase I study

Outi Ahokoski,^{1,2} Kerttu Irjala,³ Risto Huupponen,¹ Kaija Halonen,⁵ Eeva Salminen² & Harry Scheinin^{1,4}

¹Department of Pharmacology and Clinical Pharmacology, University of Turku, and Clinical Pharmacology Unit, Turku University Central Hospital, FIN-20520 Turku, ²Department of Oncology and Radiotherapy, ³Central Laboratory, Turku University Central Hospital, FIN-20520 Turku, Finland, ⁴Department of Anesthesiology and ⁵Anticancer Research, Orion Corporation Orion Pharma FIN-20101 Turku, Finland

Aims A novel non-steroidal competitive inhibitor of the aromatase enzyme, MPV-2213ad, was entered into an open dose-escalation study. The objective of this study was to investigate the tolerability and efficiency of this new compound with assessment of the hormonal effects after study drug administration.

Methods MPV-2213ad was given to 39 healthy male volunteers. Single increasing oral doses of 0.003, 0.03, 0.3, 3, 9, 30 and 100 mg were given to three subjects at each dose level, after which ten subjects received the 300 mg dose and eight subjects the highest 600 mg dose of MPV-2213ad.

Results Serum oestradiol levels were suppressed by 58–65% when MPV-2213ad was given at doses between 0.3 and 30 mg. A reduction in serum oestradiol levels by 83% from baseline was achieved with the 300 mg dose. No additional decrease was seen with the highest dose. The suppression lasted longer with higher doses of MPV-2213ad. After the 300 and 600 mg doses serum oestradiol returned to baseline within 4 days. Marked increases in serum concentrations of testosterone, androstenedione, 17-OH-progesterone, LH and FSH were also observed at doses between 100 and 600 mg of MPV-2213ad. The drug was well-tolerated and the adverse events were mild or moderate including hot flushes, mild vertigo, nausea, acne and gastrointestinal discomfort.

Conclusions MPV-2213ad has a potent, dose-dependent inhibitory effect on serum oestradiol. It was selective for the aromatase enzyme with no signs of adreno-cortical suppression or haematological or biochemical toxicity.

Keywords: aromatase inhibition, MPV-2213ad, Phase I study

Introduction

Inhibition of oestrogen biosynthesis by selective blockade of the aromatase enzyme has become an established method of hormonal treatment in oestrogen dependent malignant conditions. The first aromatase inhibitor, aminoglutethimide, is commonly used as second line hormonal treatment in breast cancer [1]. Aminoglutethimide, however, inhibits several enzymes involved in steroid synthesis and causes distressing adverse effects [2]. Therefore, more selective aromatase inhibitors have been searched, and in fact, several new compounds have been developed and recently introduced to clinical practice.

According to the nature of interaction with the aromatase enzyme, these compounds are divided into competitive and non-competitive ('suicide') inhibitors of the enzyme [3]. A suicide inhibitor, 4-hydroxyandrostenedione (4-OHA), was introduced as the first new aromatase inhibitor. It has been used in several clinical trials and found both potent and selective [4–6]. Of the competitive inhibitors, the non-steroidal compounds fadrozole (CGS 16949A) [7, 8] and letrozole (CGS 20267) have been rather extensively studied

[9, 10]. Among other non-steroidal aromatase inhibitors, anastrozole (ICI D1033) and vorozole (R83842) are in clinical development [11, 12]. Currently, several new drugs are undergoing clinical trials [13].

The aim of this phase I clinical trial was to evaluate the hormonal effects of MPV-2213ad in healthy males and to investigate the selectivity and tolerability of this new compound.

Preclinical data

MPV-2213ad (4-[3-(4-fluorophenyl)-2-hydroxy-1-[1, 2, 4-triazol-1-yl]propyl]benzonitrile a + d) is a new orally active non-steroidal aromatase inhibitor, which has shown high potency and selectivity in preclinical studies. In *in vitro* studies with human placental microsomes MPV-2213ad inhibited human aromatase with an IC_{50} value of 0.18–0.47 μ M. MPV-2213ad did not inhibit desmolase (cholesterol side-chain cleavage) activity *in vitro* even at concentration of 1000 μ M. Furthermore, *in vitro* studies confirmed that MPV-2213ad does not inhibit human liver cytochrome P450 enzymes. In the rat, MPV-2213ad inhibits ovarian aromatase completely, suggesting a reduction of oestrogens to castrate levels. In dimethylbenzanthracene-induced rat mammary carcinoma model, MPV-2213ad

causes regression of more than 80% of the established tumours. The hormone-dependent mammary tumours are inhibited to a level seen in ovariectomised animals (unpublished results, Orion Pharma).

Methods

Subjects and study design

The study had an open parallel-group design with escalating single doses of MPV-2213ad administered to healthy male volunteers. The following doses were given orally after an overnight fast with 200 ml of water: 0.003 mg, 0.03 mg, 0.3 mg, 3 mg, 9 mg, 30 mg, 100 mg, 300 mg and 600 mg in gelatine capsules. The lower doses were tested in three subjects and the next dose level was given only if the preceding dose level was well-tolerated. Ten volunteers received the 300 mg dose. At this point of the study the good tolerability of MPV-2213ad encouraged an escalation of the dose up to 600 mg, which was given to eight volunteers.

Thirty-nine healthy men (mean age: 24 years, range 19–32 years, mean body weight 74 kg, range 62–89 kg) were recruited into the study. Their general health was ascertained by a thorough physical examination by a physician, a 12-lead ECG and the following laboratory determinations: plasma sodium, potassium, calcium, serum phosphate, urea, creatinine, albumin, total protein, total bilirubin, triglycerides, cholesterol, gamma-glutamyl transferase, aspartate transferase, alkaline phosphatase, lactate dehydrogenase, creatinine kinase, amylase, blood erythrocytes, haemoglobin, white cell count with differential count and platelets and urinalysis. The volunteers were required to keep their normal dietary habits including salt intake during the whole study period and to abstain from smoking, any use of alcohol or strenuous physical activity during the testing days. Any use of medication was prohibited for 2 weeks prior to the study. MPV-2213ad was manufactured by Orion Corporation, Orion Pharma, Turku, Finland.

The study was conducted at the Department of Clinical Pharmacology, University of Turku after a prior approval by the Joint Ethical Committee of the Turku University and the Turku University Central Hospital. The Finnish National Agency for Medicines was notified prior to the start of the study according to the national regulations. Written informed consent was obtained from every subject. The study was carried out according to Good Clinical Practice guidelines.

Experimental procedures

Blood samples for the measurements of serum oestradiol (E2), oestrone (E1), testosterone, androstenedione, 17-hydroxyprogesterone, dehydroepiandrosteronesulphate (DHEAS), sex hormone binding globuline (SHBG), luteinizing hormone (LH), follicle stimulating hormone (FSH), cortisol, prolactin, thyroid stimulating hormone (TSH), human growth hormone (hGH) and plasma renin activity (PRA) were collected prior to the test drug intake and 2, 4, 8, 24 h and 2, 4, 7, 14 and 21 days after drug administration. After the 300 mg dose, additional samples

were collected at 12 h from all ten subjects and at 16 and 20 h from seven subjects. After the 600 mg dose, these additional blood samples were collected from all eight subjects. Samples during the first study day were taken through an indwelling cannula inserted into a forearm vein. Other blood samples were drawn by separate vein punctures. Samples for PRA were drawn into EDTA tubes, allowed to clot and centrifuged at $3000 \text{ rev min}^{-1}$ at $+4^\circ \text{C}$ for 10 min. All other blood samples were drawn into glass tubes without additives. The sera and plasma were kept frozen at -20°C until analyzed.

A 12-lead ECG was recorded at screening and on days 1 and 7 after drug administration. Blood pressure and heart rate were registered before dosing and 2, 4, 8 and 24 h after drug intake. ECG with precordial electrodes was frequently monitored during the first 8 h after drug intake. Screening blood samples and urinalysis incorporated 1 and 7 days after study drug administration.

The subjects were followed in the laboratory for 8 h (after 600 mg for 24 h) after drug administration. During the observation all volunteers were allowed to move freely but not to leave the laboratory. They were required to be in a sitting position 15 min before each blood sampling. Subjective tolerability was recorded by indirect questioning during the study day and on clinical visits on days 1, 7 and 21.

Assays

Blood cell counts including white blood cells with differential count were analyzed with Technicon H2 (Tarrytown, NY, USA); sodium, potassium and calcium with flame photometry Eppendorf Efix® (Netheler-Hinz GmbH, Germany) and other chemical analyses with Hitachi 717 (Boehringer Mannheim, Tokyo, Japan).

E2, SHBG, testosterone and cortisol were analyzed with Spectria® coated tube r.i.a. (^{125}I) (Orion Diagnostica, Helsinki, Finland). E1, 17-hydroxyprogesterone and aldosterone were measured by using r.i.a. kits from ICN Biochemicals, Inc., Costa Mesa, Ca, USA. LH, FSH and PRL were measured with Autodelphia® using IFMA-kits from Wallac, Turku, Finland. TSH was analyzed with IRMA-kits from Orion Diagnostica, Helsinki, Finland. DHEAS was measured with Clinical assays (Sourin Biomedica, Saluggia, Italy). Androstenedione was measured after extraction with iso-octane using r.i.a. with dextran-charcoal separation and with androstenedione antiserum (Steranti Research Ltd, London, UK) and tritiated androstenedione (Amersham International, Buckinghamshire, UK). The analyses of PRA were performed with IRMA Pasteur kit from ERIA Diagnostics Pasteur, Marnes-la-Coquette, France. Analyses were carried out according to the manufacturers' guidelines. The sensitivities and inter-assay coefficients of variation (CV%) for the assay methods are presented in Table 3.

Statistical analyses

Formal statistical analysis was performed only after the 300 and 600 mg doses of the drug. Analysis of variance for repeated measures (RM ANOVA), with one between (treatment) and one within (time) factor, was used. Only

the predetermined primary variables (E2, testosterone and cortisol) were, however, tested. If there was a statistically significant treatment-by-time interaction, the dose levels were analyzed separately. If only the time effect was significant, contrasts for marginal means between baseline and time points were tested. Statistical analyses were performed at a two-sided 0.05 significance level.

Results

Hormonal effects

MPV-2213ad induced a dose-dependent decrease in serum E2 levels (Table 1). At the lowest dose levels, however, the effects were not consistent. In fact, after the 0.003 and 0.03 mg doses, serum E2 increased during the first 4 h. The maximal average reduction was 58% after the 0.3 mg and 83% after the 300 mg dose, nadirs being reached at 8 and 20 h, respectively. With the highest dose, 600 mg of MPV-2213ad, no additional decrease of E2 was observed when compared with the 300 mg dose. The mean absolute reduction of serum E2 in the 300 mg group was 90 pmol l^{-1} (Table 1). The duration of E2 suppression lasted longer after higher MPV-2213ad doses. After the 100, 300 and 600 mg doses the suppression persisted for at least 2 days. Statistical analyses revealed significant changes ($P < 0.001$) from baseline in the 300 and 600 mg groups already after 2 h after study drug administration, but there were no differences between the groups.

Serum E1 levels were only slightly influenced by MPV-2213ad. With the three highest doses, 100, 300 and 600 mg, a modest suppression of 19–29% from baseline was detected within 8 h. The suppression lasted approximately for 4 days.

The mean serum concentrations of testosterone, 17-hydroxyprogesterone, FSH and LH after 100, 300 and 600 mg of MPV-2213ad are presented in Table 2. Serum testosterone levels showed biphasic changes after MPV-2213ad (Table 2). An initial decline (nadir at 8 h) was followed by a dose-dependent increase which declined after

4 days but persisted above baseline at 21 days after higher doses. The highest increase (71%), however, was observed in the 100 mg group. At the highest dose level (600 mg), the increase was 42%. Statistical analyses revealed significant changes ($P < 0.001$) both in the 300 and 600 mg groups until day 21, but there were no significant differences between the two groups. Marked or moderate increases in serum LH (100–128%) and FSH (26–39%) were detected 24–48 h after the three highest doses of MPV-2213ad. Slight elevations (26–39%) in serum 17-hydroxyprogesterone and androstenedione were seen after the 300 and 600 mg doses 24–48 h after drug administration, the values returning to baseline within 1–2 weeks.

A diurnal decrease in serum cortisol (53% from baseline levels) (Table 3) and in serum aldosterone (69% from baseline levels) concentrations were seen during the day after drug administration but the changes were completely normalized in the next morning. No clear drug-related changes were seen in serum DHEAS, TSH, prolactin, hGH or SHBG levels after the 100, 300 mg and 600 mg doses of MPV-2213ad (data not shown). Therefore, the levels of these hormones were not measured from samples collected at the lower doses of the study drug. PRA levels were, however, slightly or moderately elevated in the subsequent morning values but remained within the normal range.

There were no signs of drug effects on any other assays measured in this study.

Tolerability

MPV-2213ad was generally well-tolerated and subjective adverse effects were infrequent. No clinically significant changes in blood pressure, heart rate or ECG recordings were observed. All adverse events were mild, moderate and transient. In one subject, who received the lowest dose, 0.003 mg, an abnormal elevation of creatinine kinase was observed 24 h after drug intake, but it was most probably due to strenuous physical activity, revealed by the subject, although against the protocol recommendations. Headache was reported in four subjects of which only one had

Table 1 Mean (s.d.) serum E2 levels after administration of a single dose of MPV-2213ad, expressed in pmol l^{-1} .

	MPV-2213ad								
	0.003 mg	0.03 mg	0.3 mg	3 mg	9 mg	30 mg	100 mg	300 mg	600 mg
n	3	3	3	3	3	3	3	7–10	8
0 h	101(17)	124(58)	97(20)	92(11)	155(16)	159(53)	116(32)	115(30)	101(44)
2 h	134(16)	138(72)	85(14)	82(16)	133(25)	122(48)	117(33)	73(23)	56(33)
4 h	144(19)	140(43)	70(16)	70(18)	102(13)	104(55)	86(23)	60(19)	46(27)
8 h	89(22)	89(43)	41(12)	37(13)	55(8)	57(32)	47(23)	28(9)	29(17)
12 h								25(12)	23(14)
16 h								21(9)	24(13)
20 h								21(10)	24(13)
day 1	116(45)	133(64)	85(16)	39(6)	69(3)	58(20)	36(13)	33(13)	33(17)
day 2	164(23)	124(67)	95(39)	89(10)	149(23)	130(39)	58(12)	55(18)	51(29)
day 4	114(16)	126(34)	90(34)	114(17)	126(16)	196(41)	142(55)	146(34)	112(49)
day 7	117(34)	128(41)	118(38)	104(11)	133(17)	172(23)	149(25)	125(31)	129(78)
day 14	178(76)	123(73)	134(19)	127(23)	159(13)	178(19)	163(66)	129(56)	106(45)
day 21	187(72)	124(2)	73(12)	118(42)	184(54)	150(37)	122(50)	127(56)	108(43)

	Before dosing	Day 1	Day 2	Day 4	Day 7
<i>100 mg n = 3</i>					
Testosterone	14(5.5)	17(7.0)	21(6.0)	24(6.5)	22(10.1)
17-OH-progesterone	5.4(1.8)	5.5(1.5)	5.7(1.5)	5.4(1.1)	4.9(1.6)
Androstenedione	6.9(1.9)	7.0(2.9)	6.9(2.3)	7.1(1.9)	7.5(2.0)
FSH	2.2(0.8)	2.7(1.0)	3.6(1.2)	3.5(1.3)	2.5(1.0)
LH	3.7(0.3)	5.1(1.2)	7.3(2.4)	4.4(0.7)	3.3(0.8)
Aldosterone	217(122)	217(112)	166(56)	276(5.6)	321(313)
Cortisol	770(76.8)	699(85.9)	576(44.3)	728(55.8)	713(78.1)
Oestrone	861(50.5)	793(71.7)	778(102)	805(112)	907(164)
<i>300 mg n = 10</i>					
Testosterone	17(3.3)	24(4.3)	24(4.1)	27(4.9)	22(5.2)
17-OH-progesterone	4.4(1.6)	5.8(1.4)	6.1(1.3)	5.6(1.2)	4.6(1.1)
Androstenedione	5.9(1.2)	7.0(1.4)	7.3(2.0)	7.9(1.7)	6.3(1.1)
FSH	3.1(0.9)	4.2(0.8)	5.5(1.5)	5.0(1.1)	3.3(0.8)
LH	3.1(1.4)	6.7(1.7)	7.0(2.0)	5.5(1.6)	4.0(1.5)
Aldosterone	207(146)	300(192)	372(209)	324(200)	264(226)
Cortisol	533(145)	682(68.8)	647(107)	690(90.5)	595(160)
Oestrone	1080(249)	980(251)	943(239)	1058(251)	1080(201)
<i>600 mg n = 8</i>					
Testosterone	18(5.1)	23(6.6)	24(8.7)	26(5.2)	25(9.1)
17-OH-progesterone	5.1(1.5)	6.3(1.2)	5.4(0.9)	5.5(1.2)	5.1(1.3)
Androstenedione	6.7(2.1)	7.9(1.4)	7.3(1.5)	7.4(1.1)	7.3(1.3)
FSH	2.9(0.8)	3.8(1.2)	4.7(1.6)	4.7(1.5)	3.1(0.9)
LH	3.9(1.6)	7.8(3.7)	7.4(3.4)	5.8(2.1)	3.3(1.2)
Aldosterone	270(197)	447(205)	434(302)	297(180)	297(179)
Cortisol	657(181)	738(67.9)	648(56.1)	622(52.6)	647(98.2)
Oestrone	1034(238)	873(199)	896(251)	950(246)	973(205)

Table 2 Mean (s.d.) serum levels of testosterone, 17-OH-progesterone, androstenedione, cortisol (expressed in nmol l⁻¹), aldosterone (pmol l⁻¹), FSH, LH and E1 (international units l⁻¹) after administration of a single dose of MPV-2213ad.

Assay	Sensitivity	Inter-assay precision		
		CV%	n	Mean concentration
E2	5 pmol l ⁻¹	5.3	22	136 pmol l ⁻¹
Testosterone	0.1 nmol l ⁻¹	6.9	22	21.6 nmol l ⁻¹
Cortisol	4 nmol l ⁻¹	6.5	46	836 nmol l ⁻¹
E1	10 pmol l ⁻¹	11.0	17	827 pmol l ⁻¹
Aldosterone	1 nmol l ⁻¹	7.1	24	22 nmol l ⁻¹
17-OH-progesterone	0.3 nmol l ⁻¹	8.0	22	3.4 nmol l ⁻¹
Androstenedione	0.05 nmol l ⁻¹	11.0	28	3.8 nmol l ⁻¹
DHEAS	0.3 µmol l ⁻¹	8.6	34	5.3 µmol l ⁻¹
LH	0.05 U l ⁻¹	4.5	56	0.4 U l ⁻¹
FSH	0.05 U l ⁻¹	3.3	55	0.8 U l ⁻¹
TSH	0.005 mU l ⁻¹	5.0	41	3.4 mU l ⁻¹
Prolactin	0.04 µg l ⁻¹	2.8	30	5.9 µg l ⁻¹
hGH	0.01 µg l ⁻¹	4.3	16	2.0 µg l ⁻¹
SHBG	0.5 nmol l ⁻¹	7.0	13	10 nmol l ⁻¹
Renin	1 ng l ⁻¹	4.2	12	70 ng l ⁻¹

Table 3 The sensitivities and inter-assay coefficients of variation (CV%) for the assay methods.

recurrent symptoms. Hot flushes were recorded in two subjects, one in the 9 mg and one in the 300 mg group. This latter subject had also mild nausea, dizziness and gastrointestinal cramps during the 24 h after drug administration. One subject in the 100 mg group reported mild acne 2–3 days after the administration of MPV-2213ad. One subject, who received the 600 mg dose, had recurrent vertigo during almost 3 weeks. On physical examination, however, no abnormalities were found and on his last visit his symptoms had disappeared. In the 600 mg group mild

gastrointestinal cramps and diarrhoea were reported by three subjects.

One subject in this group, a 23-year old man, presented with malignant non-Hodgkin lymphoma Stage III B 4 months after attending the study. During the 3 week study period all his basic blood values including haematology and blood chemistry were normal. A retrospective analysis of his blood serum, collected and stored before drug intake, revealed a clearly elevated thymidine kinase value, suggesting abnormal cell proliferative activity already before entering the

study. In a thorough evaluation of this event, no connection to the study drug intake could be verified.

No clinically relevant changes in basic blood chemistry or haematology were observed in any of the participating subjects.

Discussion

MPV-2213ad is a new, competitive, non-steroidal aromatase inhibitor, which has shown high selectivity and specificity in preclinical studies. In the present study, MPV-2213ad, administered as a single oral dose to healthy male volunteers, was found to be well-tolerated, potent and a specific inhibitor of the aromatase enzyme.

MPV-2213ad appears to have a very potent dose-dependent inhibitory effect on serum E2. Within the dose range of 0.3–30 mg a 58–65% decrease in serum E2 levels was observed. The maximum degree of serum E2 inhibition (83% from baseline) was observed after the 300 mg dose 20 h after drug administration. Elevation of the MPV-2213ad dose to 600 mg had no further effect on serum E2. The maximum reduction in serum E2 is comparable with the effect of other aromatase inhibitors. For example 10 and 30 mg doses of CGS 20267 induced a 80–90% suppression in serum E2 [14]. In a Phase I study where male volunteers received single oral doses (0.1 to 30 mg) of anastrozole (ICI D1033), maximum serum E2 suppression occurred at doses of 7.5 to 30 mg [11]. After administration of MPV-2213ad, the reduction of serum E2 levels persisted for more than 24 h with doses from 3 to 600 mg. With the three highest doses of MPV-2213ad the recovery of serum E2 to baseline levels was seen within 4 days after the highest doses, showing a long-acting inhibitory effect on the aromatase enzyme comparable with other competitive inhibitors [11, 14].

Non-steroidal competitive inhibitors of the aromatase enzyme have the propensity to affect several cytochrome P-450-mediated steroid hydroxylations [15]. In previous studies with fadrozole, the observed elevation of precursor steroids such as 17-hydroxyprogesterone and androstenedione was interpreted to reflect a blockade of 11 β -hydroxylase activity with suppressive influence on aldosterone [7, 8]. Increases in serum LH, FSH and testosterone were also observed in the study with CGS 20267, but 17-hydroxyprogesterone and androstenedione were not measured in that study [9]. In our present study with MPV-2213ad, increases of serum testosterone, 17-hydroxyprogesterone and androstenedione were observed. These changes suggest a specific inhibition of the aromatase enzyme with a feed-back influence on the precursor hormones. The basal serum levels of aldosterone and cortisol did not change during dose escalation, indicating that MPV-2213ad is not blocking the hydroxylase enzymes. The alterations in serum aldosterone and cortisol concentrations during the first 24 h after drug administration probably reflect the normal diurnal pattern of these hormones [16] and no decrease was noted in their subsequent morning levels. In another recent study, we observed a similar diurnal variation in serum cortisol and aldosterone levels after placebo (data on file, Orion Pharma 1994). MPV-2213ad may be more specific than fadrozole, which was found to decrease serum aldosterone levels after high doses [14].

The inability of MPV-2213ad to decrease serum E1 in this study should be interpreted with caution since the assay sensitivity is different in various studies. In 4-hydroxyandrostenedione-treated patients, plasma E1 has been difficult to measure by immunoassay, while subsequent analysis with mass spectrometry, however, demonstrated a decrease in plasma E1 [17].

MPV-2213ad was well-tolerated at lower dose levels. After the highest dose (600 mg), gastrointestinal symptoms occurred more frequently. Main adverse effects in healthy subjects in studies with CGS 20267 have been headache and migraine; a drop in blood pressure has also been reported [9]. Four of the subjects attending this study with MPV-2213ad complained of mild headache, but no relevant changes in blood pressure were detected. This pattern of adverse events predicts good clinical tolerability of MPV-2213ad.

This phase I study introduces a new aromatase inhibitor MPV-2213ad, and indicates that it is a well-tolerated compound with a clear serum E2 lowering effect.

We gratefully acknowledge the assistance of Taina Lehti and Elina Kahra at the Department of Pharmacology and Clinical Pharmacology, University of Turku. This study was supported by Orion Corporation Orion Pharma, Turku, Finland.

References

- 1 Santen RJ, Manni A, Harvey H, Redmond C. Endocrine treatment of breast cancer in women. *Endocr Rev* 1990; **11**: 221–259.
- 2 Santen RJ, Worgul TJ, Lipton A. Aminoglutethimide as treatment of postmenopausal women with advanced breast carcinoma. *Ann Intern Med* 1982; **96**: 94–101.
- 3 Santen RJ. Recent progress in the development of aromatase inhibitors. *J Steroid Biochem* 1990; **37**: 1029–1035.
- 4 Brodie AMH, Banks PK, Inkster SE, Dowsett M, Coombes RC. Aromatase inhibitors and hormone-dependent cancers. *J Steroid Biochem* 1990; **37**: 327–333.
- 5 Brodie AMH, Dowsett M, Coombes RC. Aromatase inhibitors as new endocrine therapy for breast cancer. In *Endocrine Therapies in Breast and Prostate Cancer*. Vol. 4 ed. Osborne, CK, Boston: Kluwer Academic Publishers, 1990: 51–65.
- 6 Dowsett M. Aromatase inhibition: Basic concepts and the pharmacodynamics of formestane. *Ann Oncol* 1994; **5**: 3–5.
- 7 Santen RJ, Demers LM, Lynch J, *et al.* Specificity of low dose fadrozole hydrochloride (CGS 16949A) as an aromatase inhibitor. *J Clin Endocrinol Metab* 1990; **73**: 99–106.
- 8 Dowsett M, Smithers D, Moore J, *et al.* Endocrine changes with the aromatase inhibitor fadrozole hydrochloride in breast cancer. *Eur J Cancer* 1994; **30A**: 1453–1458.
- 9 Trunet PF, Mueller PH, Bhatnagar AS, Dickes I, Monnet G, White G. Open dose-finding study of a new potent and selective nonsteroidal aromatase inhibitor, CGS 20267, in healthy male subjects. *J Clin Endocrinol Metab* 1993; **77**: 319–323.
- 10 Iveson TJ, Smith IE, Ahern J, Smithers DA, Trunet PF, Dowsett M. Phase I Study of the oral nonsteroidal aromatase inhibitor CGS 20267 in healthy postmenopausal women. *J Clin Endocrinol Metab* 1993; **77**: 324–331.
- 11 Plourde PV, Dyroff M, Dukes M. Arimidex: A potent and

- selective fourth-generation aromatase inhibitor. *Breast Cancer Res Treat* 1994; **30**: 103–111.
- 12 Johnston SRD, Smith IE, Doody D, Jacobs S, Robersshaw H, Dowsett M. Clinical and endocrine effects of the oral inhibitor vorozole in postmenopausal patients with advanced breast cancer. *Cancer Res* 1994; **54**: 5875–5881.
- 13 Goss PE, Gwyn KMEH. Current perspectives on aromatase inhibitors in breast cancer. *J Clin Oncol* 1994; **12**: 2460–2470.
- 14 Trunet PF, Mueller PH, Girard F, et al. The effects of fadrozole hydrochloride on aldosterone secretion in healthy male subjects. *J Clin Endocrinol Metab* 1992; **74**: 571–576.
- 15 Santen RJ. Clinical use of aromatase inhibitors: Current data and future perspectives. *J Enzyme Inhib* 1990; **4**: 79–99.
- 16 James VHT. Adrenal steroid endocrinology—some unsolved problems. *J Steroid Biochem* 1991; **39**: 867–872.
- 17 Dowsett M, Cunningham DC, Stein R, et al. Dose-related endocrine effects and pharmacokinetics of oral and intramuscular 4-hydroxyandrostenedione in postmenopausal breast cancer patients. *Cancer Res* 1989; **49**: 1306–1312.

(Received 20 November 1996,
accepted 29 September 1997)