ANTICANCER RESEARCH *19*: 2261-2268 (1999)

Premenopausal Breast Cancer Patients Treated with a Gonadotropin-Releasing Hormone Analog Alone or in Combination with an Aromatase Inhibitor: A Comparative Endocrine Study

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Abstract. *Background: The combination of a GnRH analogue and an aromatase inhibitor can induce a complete estrogen blockade in premenopausal breast cancer patients. Material and Methods: Twenty-one premenopausal women with advanced breast cancer were randomised to receive the GnRH analog triptorelin (3.75 mg im monthly; n=10) alone or in combination with the aromatase inhibitor formestane (4-OHA, 500 mg im fortnightly; n=11) to compare the effect of both treatments on the patients' estrogenic milieu. Therefore, serum estrogen, gonadotropin and sex hormone-binding globulin (SHBG) levels were investigated before the start of treatment and subsequently over a three-month period. Results: There was a significant between-group difference in estrogen suppression during therapy. In comparison with baseline values, after four weeks of treatment the estradiol levels decreased by an average of 86.9% (95% CI, 70.5-94.2%) in the group treated with triptorelin alone and by 97.3% (95% CI, 94.1-98.8%; P=0.0422) in the combination group; the respective figures for estrone were 48.5% (95% CI, 27.5-63.5%) and 70.4% (95% CI, 52.3-81.6%; P=0.0007) and for estrone sulfate 56.7% (95% CI, 40-68.8%) and 80.5% (95% CI, 69.4-87.6%; P=0.0055). No difference was observed between the groups in terms of gonadotropin suppression; both treatment modalities led to a slight but delayed decrease in SHBG levels. Three of the patients treated with triptorelin alone experienced tumor regression compared with four patients in the combination group. No appreciable side effects of the combination therapy were observed. Conclusion: The treatment of premenopausal patients with triptorelin plus 4-OHA is feasible and leads to a much greater inhibition of main circulating estrogens than treatment with the analog alone. Since the combination of a GnRH analog and an aromatase inhibitor might potentially enhance the anti-tumor efficacy of the analog alone owing to more favorable endocrine effects, such a therapeutic approach deserves more extensive evaluation in the clinical setting.*

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Key Words: Formestane, aromatase inhibition, GnRH analog, premenopausal breast cancer.

Estrogens are the most important mitogens involved in supporting growth of hormone dependent breast cancer. However, estrogen biosynthesis is substantially different in pre- and postmenopausal patients, and the endocrine treatment options vary accordingly. It has been shown that GnRH analogs provide an effective means of decreasing circulating estrogen levels in premenopausal women, and there is evidence of tumor regression in a clinical setting (1). However, a potential drawback of this therapeutic approach is that it makes menstruating patients postmenopausal without interfering with androgen precursor aromatisation in peripheral tissues, a process that is believed to be the major source of circulating estrogen after menopause (2).

Although the role of aromatase inhibitors in the management of postmenopausal breast cancer is well established, a high degree of ovarian aromatase activity together with the compensatory endocrine loops induced by estrogen blockade have as yet prevented any meaningful sex steroid suppression by aromatase inhibitors in premenopausal patients (3). The data from studies of aminoglutethimide (AG), the first aromatase inhibitor used in clinical breast cancer, showed continuing premenopausal estrogen levels in spite of disturbed menstruation patterns, and a similar maintenance of premenopausal estrogen levels was shown with the use of formestane (4-hydroxyandrostenedione, 4-OHA), the first commercially available selective aromatase inhibitor (4-7).

It has been reported that some patients relapsing after a response to ovarian ablation may experience a further tumor remission if circulating estrogen is additionally suppressed by means of aromatase inhibition (8). Furthermore, a preliminary study has shown that the administration of 4-

OHA in combination with a GnRH analog leads to a more marked suppression of estradiol levels than the analog alone in premenopausal patients (7). Since the use of the combination would lead to less estrogen being available for supporting cancer growth, it has been suggested that such a treatment approach might provide greater tumor control than that achieved by suppressing of ovarian steroidogenesis alone.

On the basis of these considerations, we undertook the present pilot study with the aim of assessing the effect of the combination of a GnRH analog and an aromatase inhibitor on the patients' estrogenic milieu in comparison with that of the analog alone. Premenopausal women with previously untreated advanced breast cancer were therefore randomised to treatment with a slow-release formulation of triptorelin, a safe and potent agonistic analog, with or without the addition of 4-OHA (9). Although it was not our intention to compare the clinical efficacy of the two therapeutic regimens, the anti-tumor effects of each treatment were also reported.

Patients and Methods

Patients. Twenty-one consecutive unselected premenopausal patients with advanced breast cancer entered the study, which was conducted at the Medical Oncology B Division of Milan's Istituto Nazionale per lo Studio e la Cura dei Tumori. The patients were considered eligible if they had a newly diagnosed local regional recurrence or metastatic disease, a positive estrogen (ER) and/or progesterone receptor (PgR) status, and a performance status of ≤2 (ECOG scale), provided that they had not previously received any systemic therapy for metastatic disease. The patients were allowed to have received adjuvant cytotoxic chemotherapy, but not any previous endocrine adjuvant therapy. The patients were defined as premenopausal if they were actively menstruating at the time of recruitment. All pregnant patients, as well as those with life-threatening visceral disease (extensive hepatic involvement or brain dissemination or pulmonary lymphangitic spread), an estimated survival of less than six months or any pre-existing sex endocrine disorders, were excluded. A minimum three-week washout period from any corticosteroid treatment was required prior to entry into the study. None of the patients received any other form of endocrine treatment, anti-tumor treatment or drugs known to influence drug or hormone disposition during the study period. Written informed consent was obtained from all of the patients after full explanation of the protocol, which had been approved by the local bioethics committee.

One 52-year-old patient treated with the combination therapy showed a pretreatment endocrine profile suggestive of perimenopausal status. She (who achieved a partial tumor regression) had a baseline FSH value greater than 20 IU/L (the lower limit of the laboratory normal range for postmenopausal women) accompanied by low circulating estradiol level (less than 70 pmol/L), despite documentation of her menstrual period prior to the start of treatment. However, the pretreatment serum values of estrone and estrone sulfate lay within the range of concentrations observed in premenopausal women, and the patient was not excluded from the analysis.

The demographic details of the study population are shown in Table I. No patient was obese or had severely impaired hepatic and/or renal function. The treatment groups were comparable in terms of age, body weight, disease-free interval and previous adjuvant therapy. Clinical and radiological examinations were performed at the beginning of the study, after eight weeks as a first evaluation, and then every two months. In the absence of any severe adverse events, the treatment was continued as long as there was no disease progression. Tumor response was assessed according to standard WHO criteria. In order for a response to be classified as stable disease (SD), it had to have lasted for a minimum of six months. The relatively small number of patients compared in this study was in accordance with the aim of seeking to document only a large difference in estrogen suppression between the two treatment modalities.

Table I. *Main patient characteristics.*

	Treatment group	
	Triptorelin (n=10)	Triptorelin+4-OHA (n=11)
Age, yrs		
median (range)	45 (30-49)	45 (39-52)
Weight, kg		
median (range)	61 (53-68)	61 (48-81)
Disease-free interval		
<2 yrs	3	
≥2 yrs	8	8
Receptor status		
ER positive	10	6
ER negative	-	4
ER unknown	-	1
PgR positive	6	9
PgR negative	3	-
PgR unknown	1	2
Dominant disease status*		
Soft tissue	4	4
Viscera	7	7
Bone	1	7
No. of disease sites		
1	10	7
≥22	-	4
Previous adjuvant therapy*		
None	2	1
Cytotoxics	4	2
Radiotherapy	6	9

*some patients appear in more than one category

Dose and schedule. Allocation occurred according to a computer-based randomisation list which was kept blinded by the Italian Trials in Medical Oncology (I.T.M.O.) Data Management Service, and the treatment was disclosed to the physician only at the time of its initiation in each patient. The patients were given one of the two treatment regimens: an im depot formulation of triptorelin (Decapeptyl®) 3.75 mg once monthly administered alone or in combination with 4-OHA

Table II. *Geometric mean levels and 95% CIs (range) of serum estrogens at each time point and in each treatment group.*

Time on treatment (weeks)	Estrone (pmol/L)		Estradiol (pmol/L)		Estrone sulfate (pmol/L)	
	Triptorelin (n=10)	Triptorelin+4-OHA (n=11)	Triptorelin (n=10)	Triptorelin+4-OHA (n=11)	Triptorelin (n=10)	Triptorelin+4-OHA (n=11)
Baseline	217.4 (166.8-283.3)	235.7 (156.3-355.5)	156.2 (85.6-285.3)	239.7 (121.7-472.2)	1301.9 (916.6-1849.4)	1502.1 (1054.3-2140.1)
1	225.6 (162-314.1)	144.8*† (96.6-217.2)	75.7* (32.0-179)	106.7* (43.3-262.8)	1204.3* (837.7-1731.3)	993.9*§ (621.8-1588.6)
2	136.3* (98.1-189.1)	71.3*† (58.9-86.2)	26.3* (12.1-57.3)	11.6*‡ (7.2-18.7)	663.6* (436.4-1008.9)	461.1*§ (334.5-635.6)
4	111.9* (95.9-130.5)	69.8*† (58.7-83.1)	20.4* (12.5-33.3)	6.4*‡ (4.4-9.3)	563.2* (447.0-709.5)	292.8*§ (216.1-396.9)
8	115.7* (99.6-134.5)	67.4*† (51.6-88.2)	19.8* (15.0-26.0)	7.9*‡ (3.6-17.2)	548.7* (478.5-629.3)	313.1*§ (224.2-437.3)
12	119.4* (94.4-151.1)	68.3*† (54.8-85)	26.3* (18.7-37)	8.9*‡ (3.9-20.0)	651.1* (495.5-855.4)	301.2*§ (213.7-424.4)

*P < 0.0001 vs. baseline; †P < 0.001 vs. analog-treated group; ‡P < 0.05 vs. analog-treated group; §P < 0.01 vs. analog-treated group

(Lentaron®) 500 mg im every fortnight. Both drugs were injected by nurses in an out-patient setting during the first three months of treatment, and then the patients were trained to inject themselves. Whenever possible, treatment was started during the early follicular phase of the menstrual cycle.

Endocrine investigations. Blood samples for estradiol, estrone, estrone sulfate, sex hormone-binding globulin (SHBG) and gonadotropin measurements were taken at baseline (pretreatment), and 1, 2, 4, 8 and 12 weeks thereafter. Throughout the study, the samples were collected at the same time of day for each patient (between 9 and 10 a.m.) after an overnight fast and before drug administration. The serum was separated and stored at -20 °C until assay. All of the endocrine evaluations were performed by the personnel of the Nuclear Medicine Division of our Institute. The biochemists involved in the study were blinded to the treatment received by the patients until the endocrine evaluations were carried out.

The methodology for measuring serum estrogen levels has been previously described in detail (10). Briefly, three mL of serum were allowed to equilibrate with [^{3}H]estradiol (about 2200 dpm), or [^{3}H]estrone (about 2200 dpm) and [^{3}H]estrone sulfate (about 1300 dpm) (DuPont NEN, Belgium) for 1h at 37°C. Two mL 0.1M NaOH were then added to the samples for estradiol assessment, and the mixture was incubated at 65°C for 15 min. After standing at 4°C for 30 min, the samples were loaded onto preactivated C 18 Isolute 500 mg columns (Step Bio, Bologna, Italy) for solid phase extraction. For estrone and estrone sulfate, one mL 0.1M NaOH and one mL 0.1M HCl were added to previously equilibrated samples, which were then incubated and centrifuged in order to recover the upper phase for solid phase extraction. In all cases the columns were eluted with isooctane:ethylacetate (60:40 vol/vol) as solvent, the recovery from samples being ≥ 80% of estradiol, ≥ 85% of estrone and ≥ 80% of estrone sulfate. Each estrogen fraction was subsequently evaporated to dryness and then reconstituted; hormone concentrations were measured by means of RIA. Commercially available reagents from Clinical Assay Sorin (Saluggia, Italy) were used for the estradiol measurements: the sensitivity of the assay was 2.2 pmol/L; the intra- and inter-assay coefficients of variation (CVs) were respectively 6.2% and 8.6%. Estrone and estrone sulfate were measured using a highly specific antibody kindly provided by the Reproductive Medicine Unit of the C.N.R. at the University of Bologna (11, 12). The use of this antibody makes it possible to evaluate estrone sulfate concentrations by means of RIA without performing the hydrolysis step. The sensitivity of the assay was 7.3 pmol/L for estrone and 19 pmol/L for estrone sulfate; the intra- and inter-assay CVs were 8.2% and 9.1% for estrone, and 6.9% and 8.4% for estrone sulfate.

FSH, LH and SHBG levels were determined according to previously described methods (13). The gonadotropin assays had a sensitivity of 0.5 IU/L and an intra- and interassay CVs of 3.5% and 2% for FSH and 1.4% and 3% for LH. The minimum detectable dose was 0.5 nmol/L for SHBG; the intra- and interassay CVs were 4.4% and 7.3%.

For each hormone, all of the samples from the same patient were analysed in the same assay batch with all of the assays being carried out in duplicate.

Statistical methods. For the purposes of description and analysis, endocrine data were log-transformed in order to approximate a Gaussian distribution: geometric mean values and 95% confidence intervals were therefore used rather than arithmetic means.

The variation over time of each variable was analysed by adopting a mixed effect linear modelling approach, in such a way as to account for possible correlations among longitudinal measurements within the same subject (14). The treatment group, time of assessment and the time x treatment interaction were entered into the models using 0-1 indicator

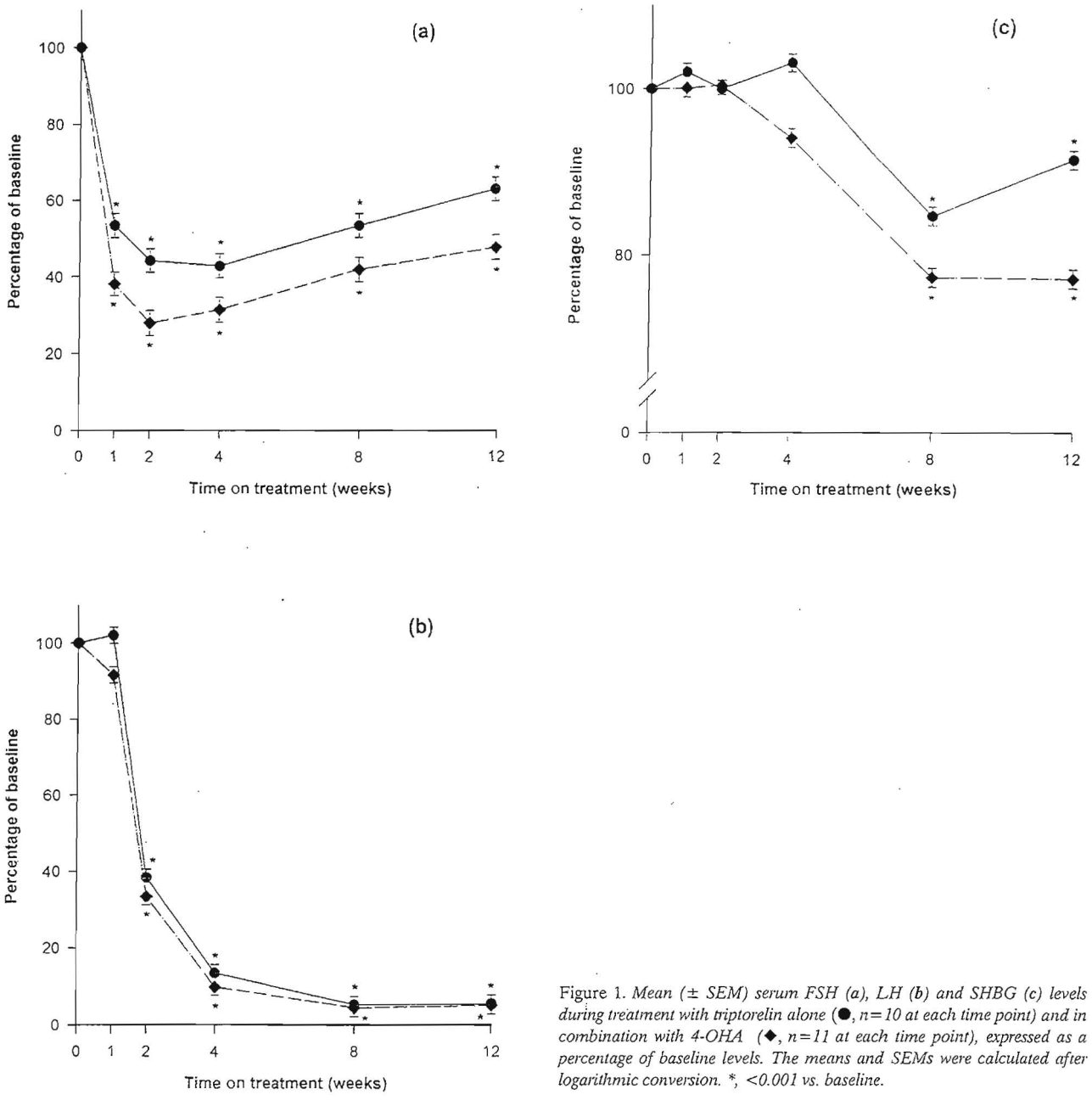

Figure 1. *Mean (± SEM) serum FSH (a), LH (b) and SHBG (c) levels during treatment with triptorelin alone (●, n=10 at each time point) and in combination with 4-OHA (◆, n=11 at each time point), expressed as a percentage of baseline levels. The means and SEMs were calculated after logarithmic conversion. *, <0.001 vs. baseline.*

variables; pretreatment measurements were also included as covariates in order to adjust for possible baseline imbalances between the two groups. A number of correlation structures between the longitudinal measurements were tried. The reported statistical results were obtained using a first-order autoregressive correlation structure with heterogeneous variances, which generally provided the best fit. As is usually recommended, the Restricted Maximum Likelihood algorithm was adopted, retaining the conventional 5% significance level (14). The computations were made using the SAS PROC MIXED procedure (15).

The mean value of percentage suppression from baseline for each hormone was calculated as 100 minus X, where X is the geometric mean value of the individual parameters at each time point in the on-treatment situation expressed as a percentage of baseline levels.

Results

Endocrine effects. The serum levels of estrone, estradiol and estrone sulfate before and during treatment in each group of patients are given in Table II. Both therapeutic modalities led to a high degree of estrogen suppression over time. In detail,

treatment with triptorelin plus 4-OHA decreased serum estradiol from premenopausal to postmenopausal levels (less than 20 pmol/L) in nine of eleven patients after two weeks of therapy compared to five of ten patients treated with the analog alone. After four weeks of treatment, the baseline estradiol levels in the monotherapy and combined groups had respectively decreased by an average of 86.9% (95% CI, 70.5-94.2%) and 97.3% (95% CI, 94.1-98.8%); estrone by an average of 48.5% (95% CI, 27.5-63.5%) and 70.4% (95% CI, 52.3-81.6%); and estrone sulfate by an average of 56.7% (95% CI, 40-68.8%) and 80.5% (95% CI, 69.4-87.6%). Only one patient in each group experienced an initial decrease in serum estradiol that fell in the postmenopausal range of values and thereafter gradually increased to the baseline level after three months of treatment.

Overall, the time x treatment interaction terms were never statistically significant, thus denoting similar trends in the two groups of patients; but significant results were obtained for the time ($P<0.0001$, always) and the treatment factors ($P=0.0422$ for estradiol, $P=0.0007$ for estrone and $P=0.0055$ for estrone sulfate). This means that although both treatments were effective in decreasing circulating estrogen levels, the combination led to a much greater suppression of each steroid.

The on-treatment concentrations of gonadotropins and SHBG are shown in Figure 1 as a percentage of baseline levels. The mean pretreatment levels of LH were respectively 5.1 IU/L (95% CI, 3.8-6.7 IU/L) and 6.7 IU/L (95% CI, 5-8.8 IU/L) in the analog alone and the combination group, and they fell to mean levels of about 3 IU/L in both groups after two weeks of therapy ($P=0.0001$). This value is in the range found during the early follicular phase of the menstrual cycle and is consistent with an effective suppression of ovarian steroidogenesis. The LH levels were close to the detection limit of the assay (0.5 IU/L) in all of the patients after the first month and remained unchanged thereafter. The pattern for FSH was different from that for LH: after two weeks of treatment, serum levels decreased from a mean baseline value of 10.4 IU/L (95% CI, 7.5-14.4 IU/L) to 4.4 IU/L (95% CI, 3.6-5.4 IU/L) in the group treated with triptorelin alone (mean suppression of 57.7%, $P=0.0001$), and from 15.3 IU/L (95% CI, 10.6-22.2 IU/L) to 4.3 IU/L (95% CI, 3.3-5.5 IU/L) in the combination group (mean suppression of 71.9%, $P=0.0001$); in both groups there was a progressive increase to mean FSH values still significantly different from baseline concentrations from month 1 onwards ($P=0.0001$). Although this trend in FSH levels to increase over time was apparent in all patients, only two and three women, respectively, in the triptorelin and combination groups showed a nearly complete recovery in circulating FSH after three months of treatment. No significant difference in the mean gonadotropin levels during treatment was observed between the two groups ($P=0.4716$ and $P=0.9649$ for FSH and LH, respectively).

Table III. *Characteristics of responders to triptorelin treatment with or without 4-OHA.*

Patient no.	Treatment group	Age at start of therapy	Disease sites	Clinical outcome	Duration of response (weeks)
10	Triptorelin	47yrs	lung	PR	72+
12	Triptorelin	46yrs	soft tissue	CR	132
19	Triptorelin	47yrs	pleura	PR	72
11	Triptorelin+4-OHA	52yrs	lung	PR	36
13	Triptorelin+4-OHA	48yrs	soft tissue	CR	96
20	Triptorelin+4-OHA	47yrs	lung	PR	88+
21	Triptorelin+4-OHA	45yrs	lung	PR	68

CR=complete tumor regression
PR=partial tumor regression

There was a slight but delayed decrease in serum SHBG levels in both groups: after two months, circulating levels in the group treated with triptorelin alone fell from a mean baseline level of 54.3 nmol/L (95% CI, 40.8-72.2 nmol/L) to 46.0 nmol/L (95% CI, 33.2-63.8 nmol/L; mean suppression of 15.3%, $P=0.0004$); in the combination group, they fell from 40.4 nmol/l (95% CI, 26.9-60.9 nmol/L) to 31.3 nmol/L (95% CI, 22.7-43.2 nmol/L; mean suppression of 22.5%, $P=0.0004$). No further reduction was subsequently observed, and there was no difference in the trend in SHBG levels between the two groups over time ($P=0.4533$).

Anti-tumor effects. Triptorelin alone led to tumor regression in three patients and SD in four; the remaining three patients progressed. In the combination group, there were four responders, three patients with SD and four with progressive disease. The clinical details of the responding patients in each group are shown in Table III. The median time to progression was 32 weeks for patients treated with the combination compared to 20 weeks for those in the triptorelin group.

Only two and three patients treated with the analog alone and the combination, respectively, started treatment more than ten days after their last menstruation. However, no patient experienced delayed amenorrhoea beyond the first month of therapy in either group. No patient in either group was withdrawn from the study because of treatment-induced side effects. As far as the systemic tolerability of the two therapeutic regimens is concerned, the main side effects were hot flushes, which occurred in a larger proportion of patients in the combination group compared with those treated with the analog alone. The im injection of 4-OHA led to injection site-related pain in only one patient but this side effect was mild and transient.

Discussion

In both groups the pretreatment values of estrone and estradiol were in the same range as those previously reported by ourselves and others, whereas the estrone sulfate levels were slightly lower than those reported for normally menstruating patients in the follicular phase of cycle (13, 16, 17). The patterns of estradiol, estrone, estrone sulfate, SHBG and gonadotropins in the patients receiving triptorelin alone were also similar to the published data on the ability of GnRH analogs to reduce circulating levels of these endocrine parameters (1, 13, 18).

In the present study, the combination of triptorelin and 4-OHA led to much greater suppression of the main circulating estrogens than did the analog alone. There is a previous report dealing with the endocrine effects of the combination of the GnRH analog buserelin and the aromatase inhibitor AG in premenopausal advanced breast cancer (19). Although details of the sensitivity of the assays were not presented in this study, the association was reported to cause no significant inhibition in gonadotropin levels, while an effective suppression of serum estradiol levels occurred in only three of five patients. However, these results were presumably due to the inability of buserelin, when administered intranasally, to effect a successful medical castration (20).

To the best of our knowledge, this is the first report of the estrone, estrone sulfate and SHBG changes in association with estradiol and gonadotropin measurements in patients treated with the combination of a GnRH analog and a selective aromatase inhibitor such as 4-OHA. So far the effects of such an association on serum estradiol levels have been studied in only five patients who were given 500 mg injections of 4-OHA at weekly intervals when they were already on treatment with goserelin (7). This study found that the addition of 4-OHA was associated with a further substantial reduction in circulating estradiol below the levels reached with goserelin alone, without any significant effect on gonadotropin concentrations; these findings are in agreement with our own results. Furthermore, although a "high-dose" drug schedule of 4-OHA was used in that study, the mean estradiol value of about 6 pmol/L observed one week after the first injection was similar to the mean levels of steroid found in the present investigation from week 4 onwards. Since the recommended clinical dose of 250 mg 4-OHA every two weeks has been reported to cause a less profound inhibition of *in vivo* aromatisation than the dose of 500 mg fortnightly, the latter dosage was selected for the present investigation (21).

One other interesting finding is that the combined treatment induced a greater suppression of estrone and estrone sulfate levels than that obtained with the analog alone, with the on-treatment concentrations of both steroids falling within the range of the values expected for naturally postmenopausal women (approximately 70 and 400 pmol/L for estrone and estrone sulfate, respectively) (22). Estrone sulfate is of particular biological interest since it may act as a reservoir for the formation of estrone via the sulfatase pathway that makes the major contribution to breast tumor estrogen synthesis (23).

The between-group difference in estrogen suppression during therapy is probably due to 4-OHA blockade of the peripheral aromatisation of circulating androgen precursors. It is worth noting that the postmenopausal ovary secretes a large amount of testosterone and a moderate amount of androstenedione, which are substrates for the aromatase enzyme (16). Moreover, a significant drop in serum estradiol levels has been demonstrated in postmenopausal women with breast cancer during treatment with goserelin (24). This observation was interpreted as being due to the reduction in high ovarian output of androgen precursors secondary to the marked analog-induced decrease in gonadotropin levels.

Although there was some inter-individual variation in estrogen suppression in the combination group, only one 44-year-old patient experienced a complete recovery in estradiol levels after three months of treatment and also showed only a minor inhibition of both serum estrone and estrone sulfate. The patient had a body weight within the normal range and showed no tumor response to treatment. Although it has been reported that circulating estrogen levels may recover in some patients as they approach two weeks from the time of their last 4-OHA injection, this hormone escape phenomenon has been observed only in patients receiving the 250 mg dose (25). At present the possibility that some patients may be less sensitive to 4-OHA is merely speculative: although it has been shown that 4-OHA may have little effect on intra-tumoral aromatase activity in a minority of patients, the results of studies measuring the *in vivo* aromatisation do not really support the hypothesis (21, 26, 27). Conversely, it might be hypothesized that ovarian steroidogenesis has contributed to the lower estrogen suppression observed in this patient, since the recovery in serum estradiol was associated with a failure to inhibit circulating FSH levels fully during therapy and FSH can stimulate the accumulation of the aromatase enzyme in ovarian tissues (28). This conclusion would be supported by the fact that there was a complete recovery of estradiol levels in association with a tendency of serum FSH to rise during therapy also in one patient treated with triptorelin alone. However, a lack of correspondence between serum FSH concentrations and ovarian estradiol production were also reported during treatment with GnRH analogs (29). Accordingly, we observed a recovery in the on-treatment FSH concentrations in a further three patients (one in the analog group and two in the combination group) whose estradiol levels fell and remained in the range of values found in postmenopausal women. It has been shown that the pituitary secretion of FSH in humans is modulated independently by estradiol and inhibin acting to inhibit, and by activin acting to increase FSH release (30). Although an insufficient ovarian production of inhibin may contribute to the recovery of FSH levels during GnRH analog therapy, it has been suggested

that the main factor accounting for this phenomenon is negative feedback to the pituitary from estradiol suppression (31, 32).

It is likely that the marked treatment-induced decrease in ovarian estrogen production is the mechanism behind the suppression of hepatic SHBG synthesis in menstruating patients (33). Indeed, a similar effect on SHBG levels has been also reported in postmenopausal patients receiving oral 4-OHA, but this decrease was not significant when the drug was given parenterally (25). Although a fall in serum SHBG may increase the free fraction of testosterone and estradiol that is available to diffuse into the tumor cell, the combination therapy did not affect binding-protein levels substantially more than did the analog alone in the short term.

In conclusion, since the major aim of endocrine therapy is to reduce endocrine stimulation of the breast tumor cell, greater suppression of the estrogenic milieu can theoretically be expected to be more effective in terms of anti-tumor activity. In this light, our results confirm and extend the endocrine data supporting the biological rationale for the use of 4-OHA in premenopausal patients simultaneously undergoing ovarian steroidogenesis inhibition, although the clinical value of such a therapeutic approach remains to be addressed in the advanced disease setting. However, it should be considered that the availability of the new and potent orally active non-steroidal aromatase inhibitors is likely to lead to these drugs being used in preference to less potent compounds such as 4-OHA in order to induce a more complete estrogen blockade (34).

Acknowledgements

The authors would like to thank the Italian Trials in Medical Oncology (I.T.M.O.) Scientific Service for its technical and editorial assistance. This study was supported by a grant from IPSEN S.p.A. (Milan, Italy).

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Received December 21, 1998
Accepted February 24, 1999