J. steroid Biochem. Vol. 23, No. 5B, pp. 867–873, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

LHRH-AGONIST TREATMENT IN CLINICAL AND EXPERIMENTAL HUMAN BREAST CANCER

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Summary—Thirty-one premenopausal patients with metastatic breast cancer were treated for 3–33 months with the potent LHRH–agonist Buserelin (Hoe 766) as a first-line therapy. Twelve women (group IA) were treated with a daily dose of 3 × 400 μg Buserelin intranasally and 10 women (group IB) with a daily dose of 2 × 1 mg subcutaneously after parenteral treatment with 3 mg per day in the first week. Nine patients (group II) were treated chronically with 3 × 400 μg Buserelin intranasally in combination with 2 × 20 mg tamoxifen ($n = 5$, group IIA) or 4 × 45 mg megestrol acetate ($n = 4$, group IIB). A great variation in hormonal response with recurrent peaks of E_2 in about half of the patients was observed in groups IA and IIA, while in group IB and IIB a "chemical castration" was reached in all patients with the most pronounced suppression of E_2 secretion in group IB. An objective tumour response was found in 13 (42%) and stable disease in 7 (23%) out of 31 patients. Nine out of 22 patients (41%) treated with Buserelin alone showed an objective response. In 8 of 17 patients (48%) with an estradiol receptor-positive tumour and in one of 2 patients with an ER-negative tumour we observed an objective remission. In an experimental study we found that Buserelin has a weak direct anti-estrogenic effect on the growth of human mammary tumour cells (MCF-7) *in vitro*. In conclusion medical treatment with high doses of Buserelin appears as effective as surgical castration in premenopausal metastatic breast cancer with an absence of serious side effects.

INTRODUCTION

Analogues of luteinizing-hormone-releasing-hormone (LHRH) are of increasing importance and of great promise in the treatment of different kinds of tumours [1–6]. In animals chronic treatment with pharmacological doses have been reported to cause exhaustion and desensitization of gonadotroph cells in the pituitary, inhibition of prolactin secretion, "chemical castration" with a striking fall in plasma sex steroids followed by a reduction in weight of accessory sex organs, inhibition of enzymes involved in steroidogenesis, and antagonism of biological actions of sex steroids [7, 8]. Furthermore, the findings of both LHRH-like receptors in an experimental prostate tumour [9] and the observation of an inhibitory effect of a LHRH analogue on the growth of mouse mammary tumour cells *in vitro* [2] suggest a possible direct effect of LHRH analogues at the level of hormone dependent tumour cells.

During the last 2–3 years analogues of LHRH have been shown to cause long-term remissions of prostate tumours in a number of clinical studies [1, 10–12]. However, reports on the effects in patients with breast cancer are limited [13]. Since 1981, we have been working on the antitumor effects of LHRH-agonist Buserelin (Hoe-766) for the treatment of metastatic breast cancer. In addition, the effect of a combination therapy of this LHRH agonist with tamoxifen and megestrol acetate was investigated especially with respect to the endocrine response. Based on our previous reports [13–16] we present this review on long-term LHRH agonist treatment (3–33 months) in 31 patients and describe results of laboratory experiments on the effect of Buserelin on a human mammary tumour cell line (MCF-7).

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PATIENTS, TREATMENT AND METHODS

Clinical studies

Thirty-one premenopausal patients with metastatic breast cancer gave consent for treatment with the potent LHRH-agonist Buserelin (Hoe 766) as a single agent or in combination with other agents like tamoxifen (TAM) or megestrol acetate (MA). All patients had not been treated previously for their metastatic disease. In total, 17 patients had an estradiol receptor (ER)-positive and two an ER-negative tumour, while in 12 patients the receptor status was unknown.

During the first week all subgroups of patients were treated with a daily dose of 3 mg Buserelin parenterally (for 3–7 successive days) as described before [15, 16]. Subsequently 12 patients (group IA) were treated chronically with 3 × 400 μg Buserelin intranasally (i.n.) and 10 patients (group IB) with 2 × 1 mg Buserelin subcutaneously (s.c.) decreasing this dose after 2 months with 2 × 0.1 mg per day for 1 month. Ultimately the patients in this subgroup IB have been treated with doses between 800 and 2000 μg per day s.c. Five women (group IIA) were

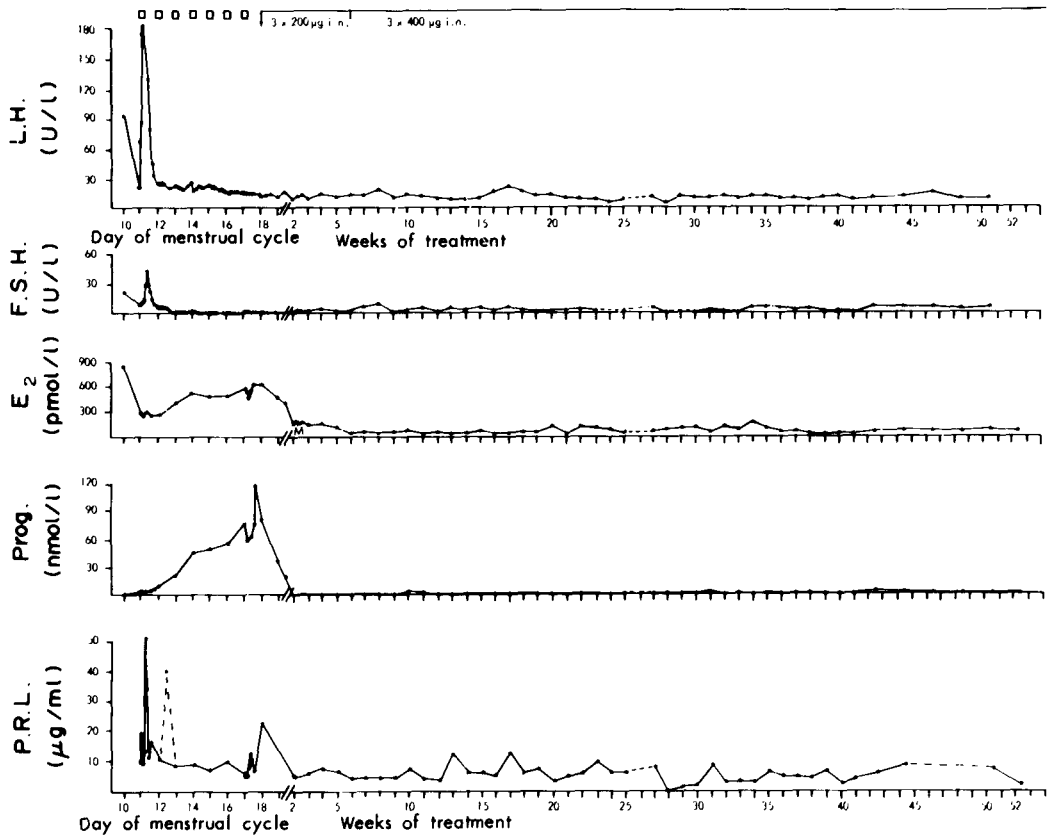

Fig. 1. Changes of plasma hormone concentrations during chronic LHRH-agonist treatment in a patient with chemical castration and a complete tumour remission during 2 years.

treated with 3 × 400 µg Buserelin i.n. in combination with 2 × 20 mg tamoxifen from the start of treatment, while in 9 out of the 12 patients of group IA tamoxifen was added later because of tumour progression or recurrent peaks of plasma estradiol (E_2). Four patients (group IIB) were treated with 3 × 400 µg Buserelin i.n. in combination with 4 × 45 mg megestrol acetate s.c.

Blood sampling, measurement of plasma luteinising hormone (LH) follicle stimulating hormone (LH) follicle stimulating hormone (FSH), estradiol, progesterone (Prog) prolactin (PRL) and estradiol receptor were done as described previously [17, 18]. Measurement of tumour response were performed according to the UICC criteria.

Significances of differences between mean values at various time points within treatment groups were assessed by Student's paired *t*-test.

Experimental studies

MCF-7 cells cultured at 37°C in an atmosphere of 5% CO_2 were trypsinized and seeded in T-25 flasks in "fully supplemented medium" to allow attachment of the cells to the culture flasks [19, 20]. Additions to the medium included estradiol, the LHRH agonist Buserelin, and synthetic LHRH (Relefact, Hoechst AG). These additives were given either alone or in combination at various concentrations.

In some experiments dextran-coated charcoal treated foetal calf serum (DCCFCS) was used. After the desired culture period the medium was removed and the cells were washed twice with 0.154 M NaCl. Thereafter, cells were dissolved in 1 ml 1 M NaOH at 50°C for 1 h. The protein concentration of the resulting solution and the DNA content were measured as described elsewhere [20]. An excellent correlation between DNA and protein contents was observed (0.863, $P < 0.001$).

RESULTS

Clinical studies

Endocrine effects

Gonadotropins. On the first treatment day peak values for plasma gonadotropins were reached about 4–6 h after the start of single treatment with Buserelin. Thereafter plasma LH and FSH dropped rapidly in spite of continuous infusion with Buserelin while during the following days no significant increase was observed (Fig. 1) indicating pituitary exhaustion and desensitisation.

In group IA mean plasma LH concentrations decreased below pretreatment levels after 2–3 weeks and continued to decrease slowly (Fig. 2). Mean plasma FSH concentration fell to the pretreatment level within 3 days, decreased further during the first week and remained thereafter at the same level during

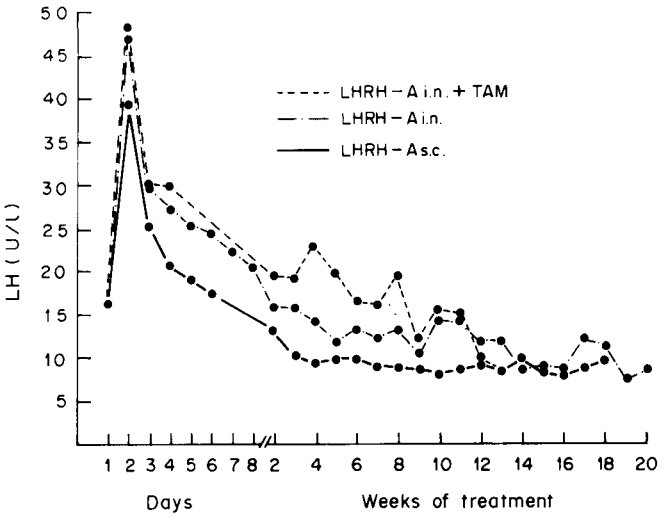

Fig. 2. Mean plasma concentrations of LH during chronic intranasal (group IA) and subcutaneous (group IB) treatment with Buserelin as a single treatment and in combination intranasally with tamoxifen (group IIA).

more than 1 year. During chronic subcutaneous treatment (group IB) the suppression of plasma LH (Fig. 2) and FSH (Fig. 3) was more pronounced than during intranasal administration. No ovulatory peaks were observed in both subgroups, while subnormal gonadotrophin levels occurred rarely.

During combination therapy with tamoxifen (group IIA) mean plasma gonadotrophin concentrations were slightly higher than during single treatment with Buserelin (Fig. 2) while preovulatory peaks of LH and FSH were observed in some patients. In group IIB (Buserelin s.c. + MA) plasma gonadotrophin concentrations were comparable with those observed in group IB.

Sex steroids. Anovulation as indicated by persisting low plasma progesterone levels occurred in all patients of groups IA, IB and IIB (Table 1); however, sub-normal peaks of progesterone were observed in 7 out of 14 patients treated with Buserelin intranasally in combination with tamoxifen.

During single intranasal therapy with Buserelin "complete medical castration" with persistent postmenopausal plasma E_2 concentrations, was reached in only 4 out of 10 evaluable patients, e.g. in the 2 patients with a complete tumour remisson during 24 (Fig. 1) and 33^+ months. In the other, 6 evaluable patients had recurring E_2 peaks of various sizes. During chronic subcutaneous treatment plasma E_2 concentrations showed a striking fall to castration

Table 1. Relative changes in plasma hormone (LH, FSH, E_2, Prog) concentrations during single LHRH agonist treatment with Buserelin (group IA + IB) and in combination with tamoxifen (TAM) or megestrol acetate (MA) (i.e. groups IIA and IIB respectively)

	LH/FSH	E_2	Prog.
IA LHRH-A i.n.	↓	↓=↑	0
IB LHRH-A s.c.	↓↓↓	↓↓↓	0
IIA LHRH-A i.n. + TAM	↓=↑	↓=↑	↓=0
IIB LHRH-A i.n. + MA	↓↓	↓↓	0

↓-reduced, ↑-increased, = -equal, 0-no change.

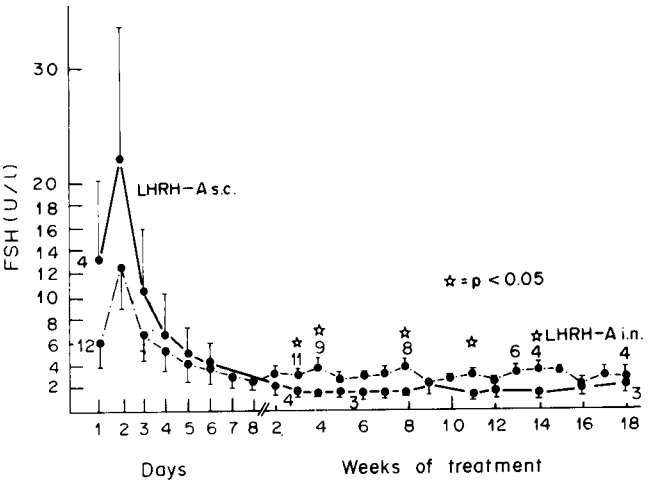

Fig. 3. Mean plasma concentrations (±SEM) of FSH during chronic intranasal (i.n.) and subcutaneous (s.c.) treatment with Buserelin alone (group IA and IB).

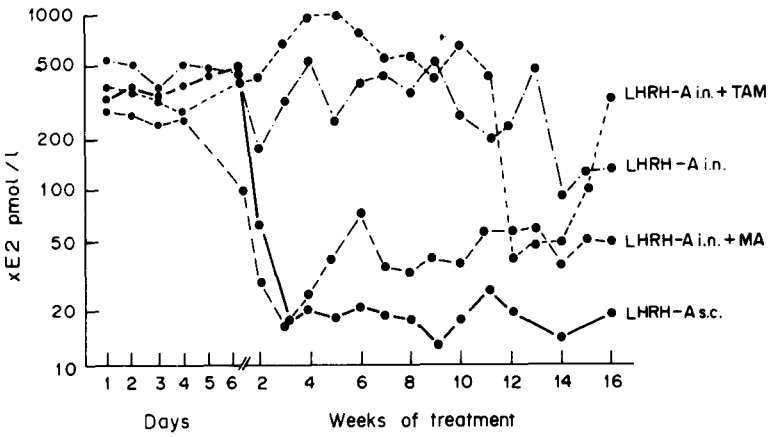

Fig. 4. Mean plasma E_2 concentrations during single treatment with Buserelin and in combination with tamoxifen (TAM) and megestrol acetate (MA); groups IA, IB, IIA and IIB.

levels within 2–3 weeks in all 10 patients (group IB) with a mean concentration of 19 pmol/l (= 5 pg/ml) during long-term treatment (Fig. 4).

The response of E_2 concentrations during combination therapy with tamoxifen was very variable; from complete medical castration level to hyperstimulation level. Recurrent peaks of E_2 occurred in 8 out of 14 treated patients. In contrast, during combination therapy with megestrol acetate no peaks of E_2 were observed, while plasma E_2 concentrations were suppressed to a mean value of 45 pmol/l (Fig. 4).

Prolactin. On the first treatment day a moderate rise of plasma PR was seen in 10 out of 11 patients of group IA. Peak values between 7 and 51 μg/l were reached mostly at 6 h after start of parenteral treatment. The mean basal value of 7.5 ± 1.12 μg/l increased to a mean peak value of 18 ± 3.9 μg/l ($P < 0.05$). On the last day of parenteral single treatment with Buserelin plasma PRL levels remained unchanged and equivalent to the pretreatment values in 9 examined patients.

During parenteral therapy the mean night peak of PRL at 0100 decreased from 27.2 ± 4.6 to 15.9 ± 3 μg/l ($n = 6$, $P < 0.05$). During chronic intranasal treatment with 3×400 μg Buserelin the day–night rhythm remained intact (Fig. 5). Pituitary PRL reserve, as measured by the TRH-test, showed an increase in 7 of 9 investigated patients after 1 week of treatment ($n = 9$, 58.7 ± 13.3 to 76.6 ± 14.1, $P < 0.05$). After 3 months there was no significant difference with pituitary PRL reserve as measured before the start of treatment.

In the group of patients treated with Buserelin in combination with tamoxifen (group IIA) only a temporary small rise in plasma PRL was seen on the first treatment day (8.4 ± 1.98 to 13.5 ± 1.93 μg/l, $P < 0.05$), which did not occur on the last day of parenteral treatment. During chronic treatment the day–night rhythm was not present in 3 investigated patients (Fig. 5). In group IIB there was a tendency to an increase in basal plasma PRL levels probably caused by megestrol acetate, which was shown to increase prolactin secretion [21].

Effects on tumour growth

In group IA an objective tumour response was found in 4 out of 12 patients with a mean duration of 16^+ months (Table 2). The longest duration of response occurred in the 2 patients with a complete remission (CR). One had a recurrence of tumour after 24 months of treatment and did not respond to tamoxifen as a second line agent; the other patient is

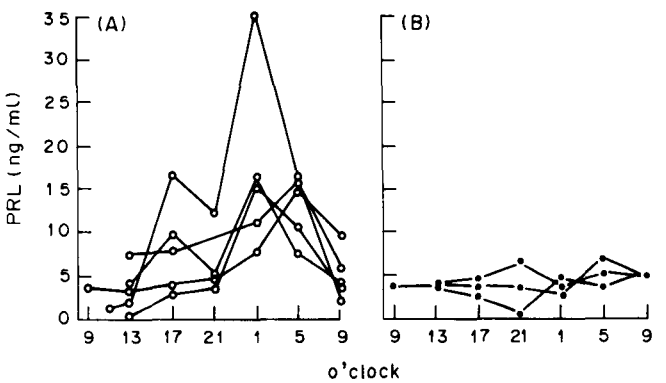

Fig. 5. 24-Hour profile of plasma prolactin (PRL) concentrations during intanasal therapy with Buserelin alone (A) and in combination with tamoxifen (B); groups IA and IIA.

Table 2. Antitumour effects in 31 premenopausal patients with metastatic breast cancer during single LHRH agonist treatment with Buserelin (group IA + IB) and in combination with tamoxifen (TAM) or megestrol acetate (MA)

Group	Treatment	CR + PR	No change	Failure	*n*
IA	Hoe 766 i.n.	4 × ($\bar{x}$ = 16$^+$ m)	4 × (3–5 m)	4 ×	12
IB	Hoe 766 s.c.	5 × ($\bar{x}$ = 5$^+$ m)	1 × (5 m)	4 × (2 × mixed)	10
IIA	Hoe 766 + TAM	3 × ($\bar{x}$ = 10$^+$ m)	0 ×	2 ×	5
IIB	Hoe 766 + MA	1 × (19$^+$ m)	2 × (17, 20$^+$)	1 ×	4
Total		13 × (42%)	7 × (23%)	11 × (35%)	31

Objective response rate during single Hoe 766 treatment (IA + B) = 9/22 (41%). Longest duration of response: 33$^+$ months.

still in complete remission after 33 months of treatment without any side effects. In group IB, with a relatively short mean duration of follow up, an objective response was observed in 5 out of 10 patients (2 × CR) with a longest duration of response of 9 months.

In the 5 patients treated with Buserelin in combination with tamoxifen (group IIA) 3 objective remissions were observed; the longest duration of response until now is more than 22 months. In the 4 patients treated with Buserelin in combination with megestrol acetate (group IIB) one partial response (19$^+$ months) and two times stable disease (Table 2) occurred. On the whole an objective tumour response was observed in 13 of all 31 patients (42%) and in 9 of 22 patients (41%) during single treatment with Buserelin (group IA + B). No side effects occurred with the exception of those caused by the intended hypogonadism.

Antitumour effects in relation to receptor status

Only the patients of group IB were selected for receptor status. Five of these ten patients (50%) with an ER-positive tumour showed an objective response. In total, 17 out of the 31 patients had an ER-positive tumour and 8 of them (47%) showed an objective response. Of the 2 patients with an ER-negative tumour 1 had a partial remission during 5 months, while 4 of the other 12 patients with an ER-unknown tumour showed an objective response also.

EXPERIMENTAL STUDIES

At concentrations of 80 and 800 nmol/l, Buserelin did not affect the growth of the MCF-7 cells significantly (Fig. 6). Although the stimulatory effect of estradiol on the cells is rather small, addition of Buserelin combined with estradiol results in a significantly lower protein and DNA content than addition of estradiol alone. The inhibitory effect of Buserelin on the protein content of MCF-7 cultures in the presence of estradiol was dose dependent. Moreover, LHRH itself also showed a slight anti-estrogenic effect. In medium containing 10% DCCFCS Buserelin appeared to inhibit the estradiol-induced increase in cellular protein and DNA. In our experience this anti-estrogenic effect of Buserelin can be counteracted by an equimolar amount of a LHRH antagonist.

DISCUSSION

In our studies with the LHRH agonist Buserelin, the response rate of tumour growth inhibition appears at least as good as other known types of ablative or additive endocrine therapy with 50%

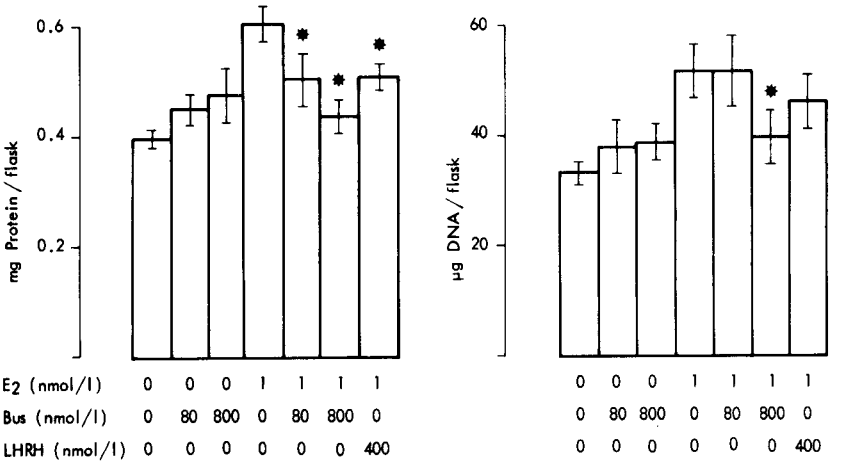

Fig. 6. Protein and DNA content of MCF-7 cultures grown in fully supplemented medium after five daily additions of estradiol (E_2) alone or combined with 80 or 800 nM Buserelin (Bus) or 400 nM synthetic LHRH. Results are given as means ± SD; n = 8–9; *P < 0.01 vs cultures kept in the presence of estradiol alone.

objective remissions in the ER-positive patients. Two recent preliminary reports concerning two studies with a relatively short follow-up confirmed our data. Harvey *et al.* [22] found an objective remission in 11 out of 25 premenopausal patients (44%) treated with 1–10 mg of the LHRH agonist Leuprolide by daily subcutaneous injection. Further, Walker *et al.*[23] reported an objective tumour response in 3 of 16 premenopausal patients treated with 250–1000 μg of the LHRH agonist Zoladex by daily subcutaneous injection.

The main mechanism of antitumour action of LHRH is probably by "chemical castration". In our clinical study the patients with a castration effect during single treatment with Buserelin showed the best responses, but it is still unclear how "complete" the chemical castration has to be. Our experimental studies with MCF-7 mammary tumour cells indicate that there may be an additional direct antitumour effect based on antagonism of the stimulatory action of estrogens remaining in the circulation after incomplete chemical castration by this new form of endocrine therapy.

With respect to the endocrine effects, the occurrence of complete chemical castration and suppression of gonadotrophin secretion appeared dose dependent. During intranasal treatment with 3×400 μg Buserelin (2% resorption, i.e. comparable with 25 μg subcutaneously) a chemical castration occurred in 4 out of 10 evaluable patients (40%) as found by Hardt and Schmidt-Gollwitzer[24] in 5 out of 9 premenopausal women (55%). Hence, in total a chemical castration was reached in 9 out of 19 patients (47%). However, high doses of Buserelin subcutaneously (800–2000 μg daily) appeared to cause very low mean plasma E_2 concentrations (as after castration) in all 10 patients (100%). During treatment with the combination of Buserelin and tamoxifen an insufficient corpus luteum function was found in half (7/14) of the patients as proven by post-ovulatory plasma progesterone levels. In some patients pre-ovulatory LH and FSH peaks were observed. This indicates that Tamoxifen may overcome the suppressive effect on hypothalamo–pituitary function following chronic intranasal LHRH-agonist treatment. Therefore we combined Buserelin with high-dose megestrol acetate treatment because of its antigonadotropic properties. From the endocrine point of view this combination appeared more suitable than the combination of Buserelin with tamoxifen. However, side effects such as body weight increase with a cushingoid face occurred after about 3 months of treatment.

In conclusion treatment with high doses of buserelin appears as effective as surgical castration in premenopausal patients with metastatic breast cancer. Apart from the absence of serious side effects another advantage of LHRH-agonist treatment might be an additional weak anti-estrogenic effect as observed on MCF-7 mammary tumour cells. A dose of 3×400 μg Buserelin i.n. (comparable with 25 μg s.c. per day) caused anovulation in all patients but suppression of follicular maturation occurred in only about half of the patients. However, high doses of Buserelin subcutaneously (2000–8000 μg) appeared to cause a striking suppression of E_2 secretion within 2–3 weeks in *all* patients.

Acknowledgements—We thank Hoechst AG, Frankfurt, F.R.G., for supplies of the LHRH agonist, the medical department of Hoechst Holland N.V. for support and fruitful comments during this study, my colleagues of the hospital Breast Cancer Group, the nurses for carefully collecting blood samples, R. Docter for hormone estimations, H. Portengen, D. M. van Gool, H. G. Bierings and P. Uitterlinden for technical assistance, Mrs A. Sugiarsi for administrative help and J. Marselje for preparing the prints.

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