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ORIGINAL ARTICLE

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Endocrine mechanism of action of toremifene at the level of the central nervous system in advanced breast cancer patients

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Abstract *Purpose*: To differentiate the antagonistic and agonistic effect of toremifene at the level of the hypothalamus-hypophysis axis a leutinizing hormone-releasing hormone (LHRH) test was performed during a phase II clinical trial. *Methods*: In 15 postmenopausal patients with advanced breast cancer, follicle-stimulating hormone (FSH) and LH release – induced by an LHRH agonist (Suprefact injection, 0.5 mg s.c.) – was monitored during a 16-week period of toremifene treatment (60 mg/day p.o.). Prolactin, estradiol, and sex hormone-binding globulin (SHBG) levels were also measured. The functional test was carried out prior to toremifene therapy and then 4, 8, 12, and 16 weeks afterward. *Results*: The drug sensitized the pituitary to the action of the gonadotrophins; the LHRH-induced FSH and LH release showed a considerably increasing tendency during the toremifene therapy. Estradiol levels decreased statistically significantly and SHBG levels showed a statistically significant increase. A decreased level of prolactin is the sign of an antiestrogenic effect of toremifene on the hypophysis and, as a result, provides evidence for a direct influence of toremifene upon the pituitary. An increase in LH and prolactin release in response to the LHRH test was characteristic in the responders. *Conclusion*: According to the LHRH test, the antagonistic effect of toremifene seems to be more dominant than the concomitantly existing agonistic property. Neither clinical nor endocrinological side effects could be observed at the level of the CNS during a prolonged period of toremifene administration.

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Abbreviations *TRH* Thyroid-stimulating hormone-releasing hormone · *HT* Hypothalamus · *HP* Hypophysis · *LHRH* Luteinizing hormone-releasing hormone · *LH* Luteinizing hormone · *FSH* Follicle-stimulating hormone · *SHBG* Sex hormone-binding globulin · *RIA* Radioimmunoassay · *IRMA* Immunoradiometric assay · *PIF* Prolactin-inhibiting factor · *PRH* Prolactin-releasing hormone

Introduction

Toremifene is an effective, safe, and easily applicable drug for the treatment of hormone-responsive cancer [5, 10, 12, 19, 20]. Due to the favorable biological and clinical activities of antiestrogens, there is increasing interest in understanding the pharmacology and biochemical mechanisms of these drugs. Although antiestrogens have been used in clinical practice since the 1970s and although their effects, especially those of tamoxifen, on serum hormone levels have been reported by several groups, their influence upon endocrine regulation at the ovarian hypothalamo-pituitary axis has not been fully clarified [1, 8, 9, 18, 27].

With regard to hormonal changes, toremifene exerts pronounced and numerous different endocrine influences upon hormone regulation in patients [7, 14–16]. In terms of the mechanism of action of the drug, it seems that toremifene has not only antiestrogenic (antagonistic) properties but a weak estrogen-like (agonistic) effect as well. In addition, a decrease in basal prolactin levels as well as in TRH-induced prolactin release suggests that toremifene also has an antiprolactinic property [16].

In the course of measuring TRH-induced prolactin release it has been established that toremifene exerts not only peripheral effects (receptor binding) but effects at the level of the hypophysis as well [16]. However, the TRH functional test is not suitable for differentiation of

the estrogen-agonistic and/or estrogen-antagonistic effect of toremifene at the level of the HT-HP axis; therefore, the performance of a functional test working at the level of the CNS is necessary. For this purpose an endocrine study, i.e., the measurement of gonadotrope hormone release – inducible by LHRH-agonist analogue injection – is suitable [17].

The goals of the present study were to evaluate the dominant endocrine effect of toremifene, antagonistic or agonistic; to investigate the LH and FSH release induced by LHRH-agonist analogue injection during a prolonged period of treatment with toremifene – this functional test enables evaluation as to how toremifene exerts its effect at the level of the HT-HP-ovarium axis; to study whether the LHRH test possesses a predictive value for patients' response in the early phase of toremifene therapy; to study prolactin secretion during toremifene administration; to analyze the modification of estradiol levels by toremifene therapy so as to control the influence of toremifene upon the HP-ovarium axis; and to control the change in SHBG concentrations so as to evaluate the intrinsic estrogenic effect of toremifene.

Patients and methods

Investigation of toremifene action at the level of the CNS necessitated the introduction of the LHRH-agonist analogue functional test (hereafter termed the LHRH test). This was the first time the LHRH test had been applied for the investigation of antiestrogen action at the level of the CNS.

The LHRH test

The principle of the LHRH test is as follows: acute administration of an LHRH agonist produces an increased release of LH and FSH. Some other hormones might be also influenced by the drug. The degree of LH and FSH release can give some information about the normal or abnormal function of the HP or about a possible hypofunction of the HT or HP [3, 4, 11, 13].

Patients' selection and inclusion criteria

Between 1991 and 1995, 15 postmenopausal women were recruited into the trial with histologically verified primary inoperable (T4) or advanced (ST IV) breast cancer. They were evaluable during a 16-week treatment period.

Patients must not have received any hormone therapy for at least 2 months or chemotherapy for at least 6 weeks before the study. They had at least one evaluable and/or measurable lesion that had shown progression during the last 3 months prior to entry into the study. The postmenopausal status required that at least 2 years had elapsed since menopause or oophorectomy. The life expectancy was more than 16 weeks. The patients had no concomitant endocrine disease and were not allowed to use any centrally acting drugs unless their clinical condition made it necessary. Patients who had failed to respond to an earlier tamoxifen treatment or had become resistant to it during a longer period of tamoxifen treatment were not included.

Performance of the study

The LHRH test was performed at a 60-mg/day oral dose level of toremifene (Fareston). The LHRH-agonist analogue (Suprefact, Hoechts) injection was applied as a single s.c. dose of 0.5 mg. The study was performed during bed rest before meals. The LHRH test was carried out prior to toremifene treatment and at 4, 8, 12, and 16 weeks after toremifene administration. Blood samples (30 ml) were taken without anticoagulant through a peripheral i.v. catheter directly prior to the Suprefact injection and at 1, 2, 3, 4, 5, 6, 24, and 48 h following Suprefact administration.

Hormonal parameters

The following hormones were measured by RIA and IRMA methods using commercial kits: LH, FSH, prolactin, estradiol, and SHBG (Orion Diagnostica, Turku, Finland).

Duration of toremifene treatment and discontinuation

The minimal duration of treatment was 16 weeks. Treatment was discontinued and patients were withdrawn from the study under the following conditions: the progress of the disease required immediate new treatment; the appearance of severe or intolerable side effects or allergic reactions; clinical reasons, judged by the investigator; and patients' refusal.

In case of an objective response (complete or partial response) or no change the treatment was continued until progression. The clinical response was evaluated as follows according to WHO criteria: CR, complete response; PR, partial response; NC, no change; PROGR or PD, progressive disease [6].

Follow-up

If the toremifene therapy seemed to be ineffective after a 16-week treatment period, administration of the drug was immediately stopped and treatment was continued with either other hormonal drugs or cytostatic agents. Follow-up examination was done every month during the endocrine investigation except for instrumental investigations, which were performed before the trial and afterward at the 16th week (at the end of the endocrine study).

Ethical considerations

All data concerning the study were handled according to the decisions of the Helsinki Declaration of the World Medical Association. This study plan was authorized by the Human Research Ethics Committee of Hungary. The patients were fully informed as to the nature of the study, its risk factors, and the right of patients to discontinue their participation in the study at any stage. Patients were asked to sign an informed consenting form.

Statistical evaluation

Student's *t*-test and Fischer's test were used for the calculation of significance [2].

Results

A total of 15 postmenopausal patients were recruited into the trial. All patients were evaluable; no patient was excluded from the trial nor was any treatment interruption necessary due to rapid progression of severe intolerance. The most common side effect was the development of or increase in hot flash (grade I–II).

The mean FSH level was in the normal postmenopausal range prior to the LHRH injection and before the start of toremifene therapy (0 week, 0 min) except for a

negligible elevation in some cases. After the administration of Suprefact a continuous increase in both FSH and LH was recorded, reaching a peak value at around 5 and 6 h, respectively, and later decreasing to the initial value. The changes in FSH levels observed during the 3- to 6-h control period were statistically highly significant as compared with the initial value (Fig. 1). At the same time, LH release showed an extremely high level of significance ($P < 0.0001$) as early as the 1st h (Fig. 2). A comparison of the mean peak values (6th h) revealed no statistically significant difference.

In the case of prolactin a marked but statistically insignificant increase appeared, with peak values being recorded at between 4 and 6 h. After 16 weeks there was a moderate suppression of the prolactin level.

A continuous decrease in mean estradiol levels was obtained during the toremifene therapy. When we compared the 0-week, 0-h and 16-week, 48-h base levels, a highly significant ($P < 0.0005$) suppression of estradiol was detected. The fluctuation in estradiol concentrations might be explained by the induced release of FSH and LH in accordance with normal endocrine regulation.

With regard to the suppression of estradiol levels by toremifene, a reduction in levels of the hormone could be seen as early as at the 4th week following toremifene administration. No value lay above the 0-week control curve in such an evaluation. This finding clearly proves the antiestrogenic effect of toremifene (Fig. 3).

A continuous and statistically significant ($P < 0.0001$) elevation in SHBG concentrations occurred over the whole period of toremifene treatment, and this increase appeared as early as the 4th week of therapy (Fig. 4).

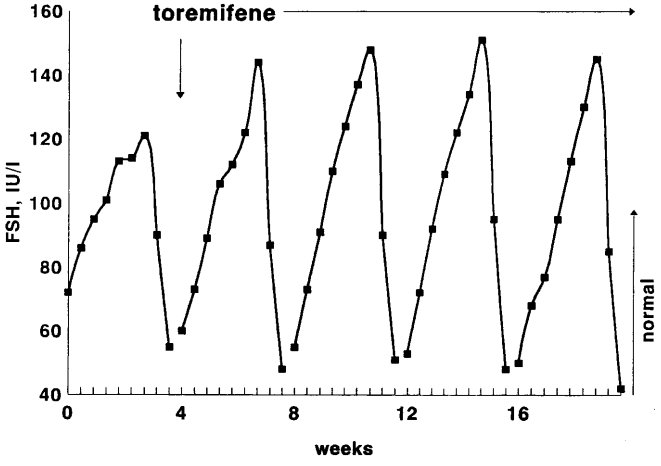

Fig. 1 Mean LHRH-induced FSH release observed in breast cancer patients ($n = 15$) relative to the normal postmenopausal range (9.4–147 IU/l). Serum FSH concentrations were measured at the indicated times (*tick marks*) prior to and after a single s.c. Suprefact injection

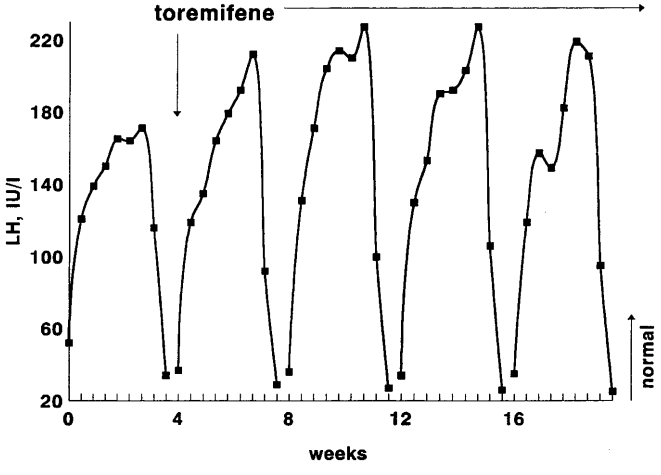

Fig. 2 Mean LHRH-induced LH release observed in breast cancer patients ($n = 15$) relative to the normal postmenopausal range (13–79.7 IU/l). Serum LH concentrations were measured at the indicated times (*tick marks*) prior to and after a single s.c. Suprefact injection

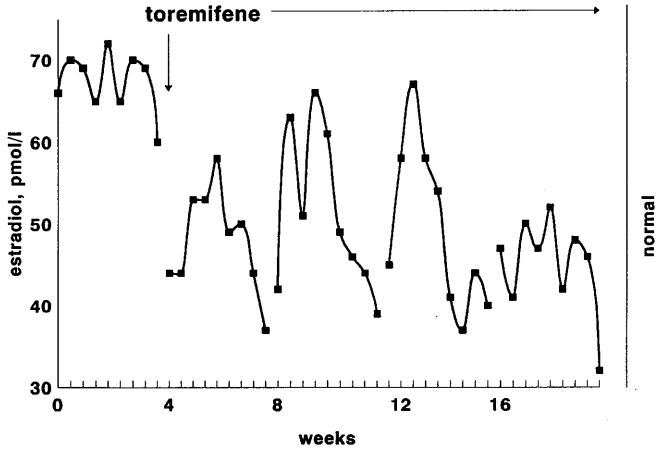

Fig. 3 Suppression of mean serum estradiol levels induced by 60 mg of toremifene treatment in breast cancer patients ($n = 15$). Serum estradiol concentrations were measured at the indicated times (*tick marks*) prior to and after a single s.c. Suprefact injection

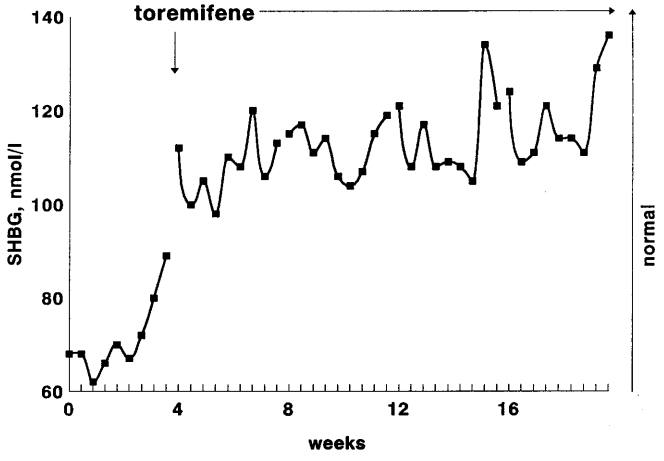

Fig. 4 Increase in mean serum SHBG levels induced by 60 mg of toremifene treatment in breast cancer patients ($n = 15$). Serum SHBG concentrations were measured at the indicated times (*tick marks*) prior to and after a single s.c. Suprefact injection

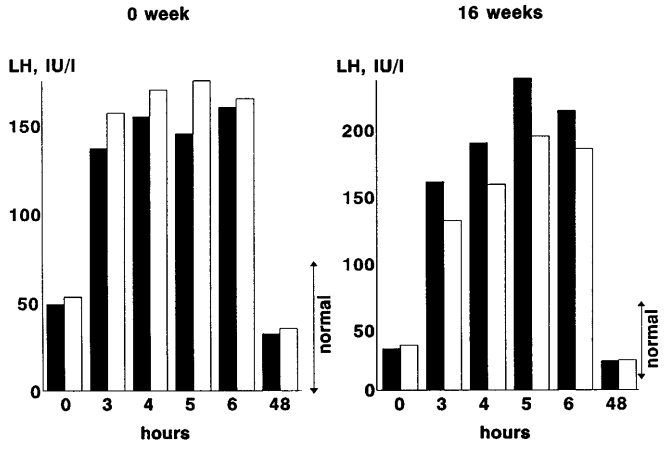

Fig. 5 Mean LHRH-induced LH release observed during toremifene treatment in responders (■, $n = 6$) and nonresponders (□, $n = 9$) relative to the normal postmenopausal range (13–79.7 IU/l)

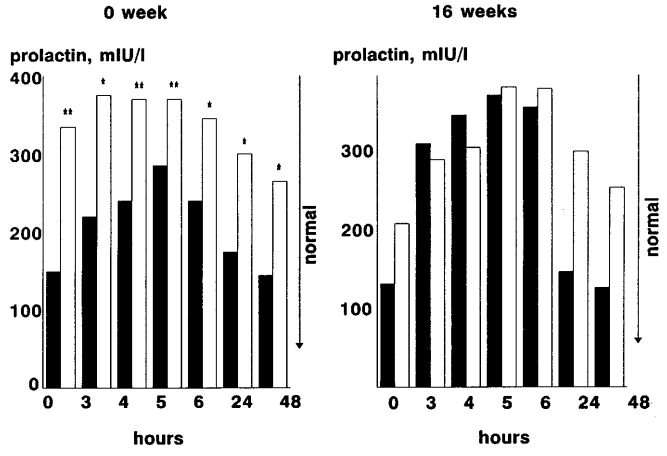

Fig. 6 Mean LHRH-induced prolactin release observed during toremifene treatment in responders (■, $n = 6$) and nonresponders (□, $n = 9$) relative to the normal postmenopausal range (50–500 mIU/l). $^{*}P < 0.05$; $^{**}P < 0.005$

The relationship between the hormonal changes and the clinical response of the patients were correlated. LH and prolactin showed some clinical response-related tendency. The base level of LH was similar in responders and in nonresponders prior to and after toremifene treatment. However, after a 16-week period of toremifene therapy, during the functional test, a more pronounced LH release occurred, albeit only in responders (Fischer's test: $P < 0.05$; Fig. 5). Changes in levels of the pituitary hormone prolactin are of clinical importance both in terms of the response and as a possible predictive parameter. Significant differences existed between responders and nonresponders in the rate of inducible prolactin release after Suprefact injection. Whereas in nonresponders there was a strong response to the LHRH test prior to toremifene therapy, in responders, higher amounts of polactin were released at the 16th week. At the same time the base level of prolactin was markedly suppressed by toremifene in responders as compared with nonresponders (Fig. 6).

Discussion

The effects of antiestrogens on gonadotrophins are complex due to opposing estrogenic actions on the HT and on the HP. Whereas high physiological concentrations of estradiol in postmenopausal patients inhibit secretion of LHRH, thus resulting in a decrease in levels of LH and FSH, exogenous and endogenous pharmacological concentrations of estrogens sensitize the pituitary gland to the action of LHRH, which tends to increase serum gonadotrophin levels. Therefore, the net antiestrogenic and/or estrogenic activity of each compound depends on the balance between these two opposing actions [1].

Using the LHRH-agonist analogue functional test, we succeeded in elucidating the action of toremifene upon the HT-HP axis. Toremifene had an antagonistic (antiestrogenic) effect; estradiol levels were statistically significantly decreased, and the base level of prolactin was also suppressed. On the other hand, toremifene had a tissue-specific agonistic (intrinsic estrogenic) activity; levels of SHBG were statistically significantly increased and those of LH were statistically significantly decreased, whereas FSH values were only moderately suppressed. Decreased levels of prolactin are the sign of an antiestrogenic effect of toremifene on HP and, as a result, provide evidence for a direct effect of toremifene on the pituitary. Toremifene did not adversely affect the normal endocrine regulation of breast cancer patients at the level of the CNS; the negative feedback mechanism was neither disturbed nor inhibited by the 16-week period of drug administration. Toremifene had an influence on the HT-HP axis; the drug sensitized the HP to the action of gonadotrophins, and the LHRH-induced FSH and LH release showed a considerable degree of increase during toremifene therapy. The fall in base levels of FSH and LH observed in postmenopausal patients during toremifene treatment may have been due to partial agonistic activity on the HT or to antiestrogenic activity on the HP.

The degree of both FSH and LH release increased continuously during toremifene therapy. One could speculate that an additive agonistic action of the two drugs is exerted or that toremifene sensitizes the pituitary gland to the LHRH stimulus due to its agonistic activity. However, after a 16-week treatment period the FSH and LH base levels were suppressed by the toremifene.

In spite of the sensitizing effect of toremifene on the LHRH-induced release of FSH and LH, the drug could normalize the hormone secretion by the end of the toremifene therapy. The slight decrease in these hormones proves the weak estrogenic (agonistic) mechanism of action of toremifene.

On the basis of the LHRH-induced LH release we concluded that the secretion rate was correlated with the clinical response of the patients; an increased response to the LHRH test during toremifene treatment was characteristic of the patients who developed a CR, a PR, or NC. In responders and in nonresponders the maximal rate of LH release recorded as the 5- to 6-h peak values at week 0 and week 16 differed significantly ($P < 0.05$, Fischer's test). It seems that a very sensitive pituitary function would be predictive of the suitability of a patient for successful toremifene treatment. In patients with PD there was a lower than normal response, if any, to LHRH injection.

According to the prolactin release induced by the LHRH test, those patients who showed high secretion curves after toremifene administration responded well to the toremifene therapy.

According to our earlier results, obtained with the TRH functional test [16], toremifene inhibited prolactin secretion at the level of prolactin-secreting cells in the pituitary gland or decreased the prolactin reserve capacity in the secreting cells. Therefore, it is tempting to speculate that one of the mechanisms of actions of toremifene may involve its antiprolactinic property in breast cancer therapy. The LHRH-agonist analogue, which exerts its effect at the HT-HP axis, can inhibit the PIF secretion or stimulate the PRH release from the HT, resulting in an increased release of prolactin from the pituitary. According to this observation on the measurement of prolactin levels after the injection of a single dose of LHRH, one could speculate as to whether this phenomenon might be a useful test to predict which patients would respond to toremifene treatment. Another hypothesis might be that patients who have a normal or a very sensitive HP function as proved by the endocrine functional tests would be candidates for successful toremifene therapy. Proof of the above-mentioned theories would require further investigations involving a larger number of patients.

The significant decrease in estradiol levels took place during toremifene administration. A similar observation has been reported by other authors [22]. On the basis of our results, it seems that toremifene has antagonistic action because it counteracted the agonistic activity of LHRH at the level of the pituitary gland.

An elevation in SHBG synthesis in the liver could be considered a potentially beneficial effect in breast cancer therapy because the unbound (and, thus, active) estradiol concentration in the serum is diminished by binding of the hormone to this transport protein.

No severe adverse reaction, including the development of second primary disease such as endometrial or liver cancer, was observed among our patients.

In summary, toremifene exerts many different endocrine effects upon hormone regulation in breast cancer patients. Despite the considerable modulation observed in the endocrine milieu, an early prediction of drug efficacy cannot be made directly on the basis of hormonal changes. According to the LHRH test, the antagonistic (antiestrogenic) effect of toremifene seems to be more dominant than the concomitantly existing agonistic (partial estrogenic) property. The LHRH test supported our earlier findings, obtained by the TRH provocation test, that toremifene exerts its effect predominantly at the level of the pituitary gland. Neither clinical nor endocrinological side effects were observed at the level of the CNS or in the periphery during a prolonged period of toremifene therapy.

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References

1. Boccardo F, Guarneri D, Rubagotti A, Casartelli GL, Bentivoglio G, Conte N, Campanella G, Gaggero G, Comelli G, Zanardi S, Nicolo G (1984) Endocrine effects of tamoxifen in postmenopausal breast cancer patients. Tumori 70: 61
2. Dixon WJ, Brown MG, Engelman L, Jennrich RI (eds) (1990) BMPD statistical software manual, University of California, Berkeley, Los Angeles, Oxford
3. Emons G, Schally AV (1994) The use of luteinizing hormone releasing hormone agonists and antagonists in gynecological cancers. Hum Reprod 7: 1364
4. Harsoulis P, Marshall JC, Kuku SF, Burke CW, London DR, Fraser TR (1973) Combined test for assessment of anterior pituitary function. BMJ 4: 326
5. Hayes DF, Van Zyl JA, Hacking A, Goedhals L, Bezwoda WR, Mailliard JA, Jones SE, Vogel CL, Berris RF, Shemano I (1995) Randomized comparison of tamoxifen and two separate doses of toremifene in postmenopausal patients with metastatic breast cancer. J Clin Oncol 13: 2556
6. Hayward JL, Carbone PP, Heuson JC, Kumaoka S, Segaloff A, Rubens RD (1977) Assessment of response to therapy in advanced breast cancer. A project of the programme on clinical oncology of the International Union Against Cancer, Geneva, Switzerland. Eur J Cancer 13: 89
7. Hindy I, Juhos É, Szántó J, Számel I (1990) Effect of toremifene in breast cancer patients. Preliminary communication. J Steroid Biochem 36: 225
8. Jordan VC (1996) How does tamoxifen work? In: Jordan VC (ed) Tamoxifen: a guide for clinicians and patients. PRR, Huntington, p 181
9. Jordan VC, Fritz NF, Gottardis MM, Mirecki DN, Ravdin PM, Welshons WV (1986) Laboratory and clinical research on the hormone dependence of breast cancer: current studies and future prospects. In: Jordan VC (ed) Estrogen/antiestrogen action and breast cancer therapy. University of Wisconsin, Madison, p 501
10. Kangas L, Nieminen AL, Blanco G, Grönroos M, Kallio S, Karjalainen A, Parilä M, Södervall M, Toivola R (1986) A new triphenylethylene compound, Fc-1157a. II. Antitumour effects. Cancer Chemother Pharmacol 17: 109
11. Kastin D, Schally AV, Gual C, Arimura A (1972) Release of LH and FSH after administration of synthetic LH-releasing hormone. J Clin Endocr Metab 34: 753
12. Pyrhönen S, Valavaara R, Vuorinen J, Hajba A (1994) High dose toremifene in advanced breast cancer resistant to or relapsed during tamoxifen treatment. Breast Cancer Res Treat 29: 223
13. Schally AV, Arimura A, Baba Y, Nai RMG, Matsuo H, Redding TW, Debeljuk L (1971) Isolation and properties of the FSH and LH-releasing hormone. Biochem Biophys Res Commun 43: 393
14. Számel I, Hindy I, Vincze B, Kerpel-Fronius S, Eckhardt S (1988) Influence of antiestrogen drugs on the sex hormone and sex hormone-binding globulin levels in breast cancer patients. Ann NY Acad Sci 538: 265

15. Számel I, Vincze B, Hindy I, Kerpel-Fronius S, Eckhardt S, Mäenpää J, Grönroos M, Kangas L, Sundquist H, Hajba A (1990) Hormonal effect of toremifene in breast cancer patients. J Steroid Biochem 36: 243
16. Számel I, Hindy I, Vincze B, Eckhardt S, Kangas L, Hajba A (1994) Influence of toremifene on the endocrine regulation in breast cancer patients. Eur J Cancer 30A: 154
17. Számel I, Hindy I, Budai B, Kangas L, Hajba A, Lammintausta R (1995) LHRH agonist analogue functional test in advanced breast cancer patients treated with toremifene. Eur J Cancer 31A: S81
18. Tellez C, Jordan VC (1995) Hormonal treatment of breast cancer. Surg Oncol Clin North Am 4: 751
19. Tomas E, Kauppila A, Blanco G, Apaja-Sarkkinen M, Laatikainen T (1995) Comparison between the effects of tamoxifen and toremifene on the uterus in postmenopausal breast cancer patients. Gynecol Oncol 59: 261
20. Valavaara R, Pyrhönen S, Heikkinen M, Rissanen P, Blanco G, Thölix E, Nordman E, Taskinen P, Holsti L, Hajba A (1988) Toremifene, a new antiestrogenic compound for treatment of advanced breast cancer. Phase II study. Eur J Cancer Clin Oncol 24: 785
21. Willis KJ, London DR, Ward HWC, Butt WR, Lynch SS, Rudd BT (1977) Recurrent breast cancer treated with antiestrogen tamoxifen: correlation between hormonal changes and clinical course. BMJ 1: 425
22. Wiseman LR, Goa KL (1997) Toremifene. A review of its pharmacological properties and clinical efficacy in the management of advanced breast cancer. Drugs 54: 141