EGF/EGF-R system and benign breast disease during and following the treatment of gynaecological pathologies with an analogue of LH-RH

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Summary: Clinical and pathological changes of the mammary gland have been studied in 64 women affected by symptomatic Benign Breast Disease (BBD) coexisting with endometriosis or uterine leiomyomata. These patients were rendered hypoestrogenic by subcutaneous administration of the LH-RH analogue Goserelin depot [D-ser (^{t}Bu)^{6} Aza-Gly^{10}-GnRH (ICI118630)] performed every 28 days, for six months. They were evaluated clinically and ultrasonographically before and after treatment to find possible changes of BBD as well as of endometriosis or uterine leiomyomata. Mammary biopsies were performed before and after treatment in all the patients to study the changes of EGF-R expression.

Results showed that clinical improvement is accompanied with a reduction of EGF-R expression.

Key words: EGF/EGF-R system; Benign Breast Disease; LH-RH analogue.

INTRODUCTION

At least 40% of premenopausal women in western countries are affected by benign breast disease (BBD), a condition that interfers seriously with normal activities in about 8% of these women ([1]).

The term benign breast disease includes several clinical, radiological and pathological entities ([2]) whose development is due to estrogen-dependent events which appear in the fertile period and regress after menopause. The extinction of estrogenic secretion accentuates the involution of glandular structures and promotes the reduction of fibrocystic lesions ([3]). BBD, fibromyomatosis and endometriosis share slightly similar etiopathogenesis in which estrogen stimulation and the epidermal growth factor / epidermal growth factor-receptor (EGF/EGF-R) system plays a crucial role ([4]).

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Estradiol induces the growth and differentiation of the mammary gland interacting with specific steroid receptors localized in the ductal and lobular epithelium. This interaction leads to an increase of receptor synthesis and to a production of some mitogenic factors, including EGF [5]. The growth of breast tissue following estrogen administration is partly related to an increased production of EGF-R m-RNA [5, 6].

EGF-R is a trans-membrane glycoprotein with protein-kinase activity [7]. The binding of ECG to its receptor exerts a strong proliferative effect on epithelial cells in vivo and in vitro [8], through a biochemical cascade which leads to DNA and RNA synthesis and, finally, to cell proliferation [9]. EGF-R expression has been immunohistochemically shown in several human tissues, such as the myometrium [10, 11, 12, 13], eutopic and ectopic endometrium [11, 13, 14, 15], endocervix [16], and mammary gland epithelium [11, 17, 18].

In the mammary gland EGF-R is expressed in different patterns in ductal and lobular epithelial and myoepithelial cells [19]. It has also been found in human milk and colostrum [20]. Probably, the EGF/EGF-R system plays a role in mammary differentiation and function involved in an autocrine-paracrine regulatory system which is a result of an equilibrium between stimulating factors such as insulin-like growth factors (IGFs) and EGF/tumor growth factor (TGF)-α, and EGF itself and inhibitory factors such as TGF-β [21].

Growth factors are also involved in the failure of growth regulation, represented by neoplastic transformation. Breast cancer cells synthesize and secrete a variety of growth factors which stimulate the progression of the primary tumor and metastases. The estrogenic dependence of some breast cancers is related to an estrogen mediated regulation of growth factor expression. Hormonal independence is due to the loss of this control, as demonstrated by the inverse relationship between EGF-R and estrogen receptor (ER) in human breast cancer cells. Both receptors rarely coexist in a primary human breast cancer, where the majority of the cells are alternately ER or EGF-R [22, 23] ositive. In non-malignant breast tissue surrounding the tumor, EGF-R and ER coexist in significant proportions, suggesting that the inverse relationship between EGF-R and ER in breast cancer may reflect an abnormal regulatory state confined to the tumor itself [24]. EGF-R positivity in breast cancer is often indicative of a bad prognosis [22, 23].

Several data available about the variations of EGF-R expression in normal and malignant breast tissues come mainly from studies of the radiolabeled EGF binding to cell membrane preparations from tissue homogenates. Rather scarce is the information about EGF-R expression in the mammary gland affected by BBD. In fibroadenomas the EGF-R was demonstrated in the ductal epithelium, myoepithelial cells and stromal fibroblasts [25]. Few studies are available about the variations of EGF-R expression in the mammary gland of women affected by BBD after hormonal therapies [26]. The hormonal drugs used for the treatment of endometriosis and uterine leiomyomata positively influence the clinical course of BBD. Danazol, gestrinone, and Gn-RH analogues determine a clinical and pathological improvement of BBD that seem to be related to a significant change of EGF-R expression [26, 27, 28].

The present study evaluates the modification of EGF-R expression in the mammary gland of women affected by BBD and treated with a LH-RH analogue because of coexisting endometriosis or uterine leiomyomata.

MATERIALS AND METHODS

Sixty-four women aged from 28 to 45 years (mean 34.7) affected by BBD with cyclical or occasionally continuous mastalgia associated with objective clinical signs of breast nodularity were recruited for the study.

They were chosen from two groups of patients affected by pelvic endometriosis and/or uterine leiomyomata, respectively.

All patients gave their informed consent to participate in the present study, which was approved by the Ethical Committee of our Institution.

Twenty women aged from 28 to 32 years (mean 29.9) were extrapolated from a group of 72 patients affected by pelvic endometriosis, whereas 44 patients aged from 30 to 45 years (mean 36.9) were chosen from a larger group of 108 women affected by uterine leiomyomata (Table 1).

Table 1. — *Patients involved in the study.*

	Total	Patients with associated symptomatic B.B.D.
Endometriosis . . .	72	20 (28%)
Fibromyomatosis . .	108	44 (41%)
	Tot. 64	

Mastalgia was evaluated by a Daily Breast Pain Chart ([29]) based on an algometric scale reporting the subjective intensity of breast pain. All the women before treatment were affected by mastalgia equal to or higher than five degrees of the algometric scale (from 1 to 10). Breast nodularity was clinically and ultrasonographically assessed. It was graded according to a nodularity score variable from 0 (no nodularity) to 3 (severe nodularity).

Goserelin depot (ICI 118630) was administered every 28 days for six months by means of a subcutaneous injection in the abdominal wall.

No patients dropped out of the study.

At the end of the treatment patients were all reevaluated to check the clinical response in terms of reduction of mastalgia and improvement of breast nodularity. Fine needle and screw-needle (Rotex H.S.) breast microbiopsies were performed before and after treatment in each patient in order to classify BBD according to Haagensen's criteria ([30]) (Table 2), to evaluate possible ultrastructural modifications of the epithelial and stromal tissues and to assess the changes of EGF-R expression in the mammary gland.

Hematoxylin-eosin staining was chosen for light microscopic observation. An electronic microscopic study was performed on samples fixed in glutaraldeide at 3% in cacodylate buffer, post-fixed in osmic acid at 1.5%, dehydrated in ethanol, transferred in ethylene oxide and then embedded in Epon.

EGF-R was immunohistochemically detected. Tissue specimens were frozen in liquid nitrogen. Cryosections, 6 μ thick, were mounted on gelatine-coated slides which were air-dried overnight at room temperature to enhance cellular morphology. Then the slides were fixed in acetone for 10 minutes at room temperature, air-dried again for 30 minutes and, finally, washed three times, for five minutes, in phosphate buffered saline solution. Endogenous peroxidase was inhibited in 3% H_2O_2 in methanol for five minutes at room temperature. Tissue samples were then incubated with anti-EGF-R monoclonal antibodies AB-3 (Oncogene Science Inc. Manhasset, N.Y.) obtained from immunization of balb/c mice and fusion of splenocytes with NS1 mouse myeloma cells. To develop the staining, slides were treated for four minutes with the enzime substrate diaminobenzidine and stained with hematoxylin.

Finally, slides were rinsed in tap water, dehydrated in ethanol and xylene, and mounted with Permount.

The intensity of staining was evaluated by two observers using a double-headed light microscope and graded as follows: 0 = no staining; 1 = light staining; 2 = moderate staining; 3 = heavy staining.

Anova and Mc Nemar chi-square tests were used for statistical evaluation of results.

Table 2. — *Haagensen's classification of B.B.D.*

Pathological characteristics.

- Adenosis
- Apocrine metaplasia
- Fibrosis
- Gross cysts
- Microcysts
- Papillomatosis
- Papilloma
- Stromal lymphocytes or histiocytes
- Sclerosing adenosis
- Stromal oedema
- Atypical lobular hyperplasia
- Fibroadenoma
- Fatty replacement
- Gross changes

RESULTS

A reduction of intensity of mastalgia occurred in all patients. Medium values were 8.3 ± 1.4 before treatment and 1.5 ± 2.2 after six months of Goserelin depot administration.

Nodularity scores decreased dramatically from 2.3 ± 0.8 to 1.6 ± 0.6 at the end of treatment. Differences were statistically significant (Anova test) (Table 3).

Light microscopic examination of breast samples demonstrated a non-proliferating mastopathy in all the women. Mastopathy was further classified as follows: prevailing cysts in 48 cases (75%); prevailing adenosis in 10 cases (16%) and prevailing fibrosis in 6 cases (9%) (Table 4).

At the end of the six month treatment the complete remission of breast pain in 40 patients (63%) and a partial remission in 14 patients (22%) was observed. No good results were obtained in 10 patients (15%).

Nodularity improved in 44 patients (69%) while it was unchanged in 20 patients (31%) (Table 5).

The best results in terms of pain relief and decrease of nodularity were obtained in the prevailing cyst mastopathy which was the most sensitive to the effect of the drug. Patients carrying a prevailing adenosis or a prevailing fibrosis mastopathy failed to show the same comforting results obtained in the cystic group (Table 6).

Breast biopsies from BBD affected women showed variable degrees of EGF-R expression before LH-RH analogue administration. Six cases showed no staining for EGF-R (8%). Eight slides were grade 1 (13%), 46 were grade 2 (73%) and four were grade 3 (6%).

After Goserelin depot treatment, 34 samples (54%) were grade 0, 18 samples showed grade 1 immunostaining (27%) and 12 samples were grade 2 (19%).

No sample showed grade 3 staining (Table 7).

Six months after the end of treatment patients were thoroughly reexamined clinically and ultrasonographically to evaluate possible relapses in terms of mastalgia as well as nodularity. Relapse of symptoms was found in 26 women. Twelve (23%) had a recurrence of mastalgia similar to the pretreatment values and 14 (31%) complained about an increase of nodularity (Table 8).

Table 3. — *Mastalgia and nodularity score before (t0) and after (tF) treatment.*

	t0	tF
– Mastalgia score . .	8.3±1.4*	1.5±2.2
– Nodularity score .	2.3±0.8**	1.6±0.6**

* p = 0.0001; ** p = 0.0001 (ANOVA test).

Table 4. — *Pathological c'assification of B.B.D.*

– Mastopathy with prev. cysts . .	48 (75%)
– Mastopathy with prev. adenosis .	10 (16%)
– Mastopathy with prev. fibrosis .	6 (9%)
Tot.	64 (100%)

Table 5. — *Mastalgia and nodu'arity: treatment results.*

MASTALGIA			NODULARITY	
Complete remission	Partial remission	No modifications	Improvement	No modification
40 (63%)	14 (22%)	10 (15%)	44 (69%)	20 (31%)

Table 6. — *Mastalgia and nodularity treatment results referring to the initial histologic table.*

	MASTALGIA			NODULARITY	
	Complete remission	Partial remission	No modification	Improvement	No modification
– Prev. cysts (n = 48) . . .	36 (76%)	8 (16%)	4 (8%)	44 (92%)	4 (8%)
– Prev. adenosis (n = 10) . .	4 (40%)	4 (40%)	2 (10%)	—	10 (100%)
– Prev. fibrosis (n = 6) . . .	—	2 (33%)	4 (67%)	—	6 (100%)

Table 7. — *Degrees of immunostaining before and after treatment with Goserelin depot.*

	t0	tF
– 0 grade . . .	6 (8%)	34 (54%)
– 1 grade . . .	8 (13%)	18 (27%)
– 2 grade . . .	46 (73%)	12 (19%)
– 3 grade . . .	4 (6%)	

t0: before treatment; tF: after treatment.
Mc Nemar chi-square test $p < 0.001$.

Table 8. — *Six month relapses related to all positive results (complete and partial remission).*

Positive results		Relapses	
Mastalgia	Nodularity	Mastalgia	Nodularity
42 (77%)	30 (69%)	12 (23%)	14 (31%)

DISCUSSION

BBD is a pre- and perimenopausal hormonal endocrine disorder [26, 31, 32, 33] that responds to hormonal treatment in most cases [18, 34, 35]. The reduction or complete inhibition of estrogenic secretion determines an improvement in subjective symptoms and objective signs of the disease, associated with an involution of the mammary gland. By inducing an hypoestrogenic hormonal milieu, through the administration of drugs such as tamoxifen [36], danazol [26, 28, 37], bromocriptine [38], Gn-RH-analogues [39, 40, 41], a reduction of estrogen dependent cell proliferation could be determined [35, 39]. Goodwin *et al.* [42] reexamining 32 studies published from 1975 to 1987 on the treatment of cyclical mastopathy and mastalgia, pointed out that bromocriptin, danazol, EP-oil, tamoxifen and a hypocaloric diet could be all regarded as effective provisions in the treatment of mastopathy [35, 38, 42]. Particularly, danazol was administered in low doses in the management of BBD limiting the treatment to the luteal phase of the menstrual cycle. The complete disappearance of breast pain was obtained in 55% of cases without relevant side effects [26, 37].

BBD is often not sensitive to the first-choice drugs or is not sensitive to any drugs at all [43]. Furthermore, about half of the patients who benefit from therapy suffer relapses as soon as the treatment is stopped [40].

The continuous research for new drugs has lead to a new approach that could be represented by LH-RH agonist administration in selected cases of chronic mastopathy refractory to other drugs [41]. The rationale of the clinical use of LH-RH analogues in the management of BBD is based on the interaction with the hypothalamic pituitary axis that causes changes in Gn-RH receptor expression and a reduction of LH and FSH endogenous synthesis [44]. LH-RH analogues cause a reduction of the basal levels of gonadotropins, loss of LH pulsatility, increase of LH/FSH ratio [45], and reduction of LH bioactive fraction [46].

The antigonadotropic action of LH-RH analogues induces complete ovarian inhi-

bition by blocking ovarian steroidogenesis ([47, 48]) and reducing the blood levels of estradiol, progesterone, prolactin and androgens, all of which are active on breast tissue in vivo as in vitro ([41, 49, 50, 51, 52]).

Monsonego *et al.* obtained an improvement of mastopathy after the LH-RH treatment, particularly in patients affected by glandular hyperplasia without atypia and negative estrogen and progesterone receptors. Therapeutical success occurred in 71% of the cases. The existence of a direct mechanism of the analogue combined with the presence of already individualized LH-RH receptors could explain the effect of the analogue in the cases of BBD negative for estrogens receptors ([41, 53]).

Hamed *et al.* showed a regression of mastalgia in 100% of patients treated with Goserelin depot. They obtained positive results in a group of patients refractory to other treatments in 56% of cases ([40]). Others demonstrated 100% remission of mastalgia after two months of Goserelin depot treatment ([41]).

In the present study nodularity improved in 44 patients (69%) whereas no changes were found in 20 cases (31%). During a second clinical control six months after the end of treatment a reappearance of mastalgia in 6 cases (23%) was found whereas nodularity returned in 14 cases (31%). A lot of studies emphasized the presence of the EGF/EGF-R system both in benign and malignant breast tissue or in endometrial and endometriotic tissue. Some papers have evaluated the reduction of EGF-R expression after hormonal therapy ([11, 25, 26]).

Lumsden *et al.* demontrated that the use of LH-RH analogues can produce a decrease of EGF binding in uterine leiomyomata but not in normal myometrial tissue, suggesting that a part of the estrogen action in promoting the growth of myomas could be mediated by EGF ([54]).

In our study 54% of the breast samples (34 cases) presented a grade 0 immunostaining at the end of treatment, while only six slides (8%) were grade 0 before treatment. Four slides (6%) were grade 3 before treatment while no sample showed the same degree at the end of therapy (Table 7).

The pharmacological effects of Goserelin depot on BBD affected mammary glands are strictly related to a marked reduction of EGF-R expression ([26]). Estrogen receptors in negative breas tissue can or cannot express EGF-R ([25]). BBD affected patients with a low EGF-R expression could have a better prognosis, if the improvement reported after LH-RH administration shows EGF-R reduction.

CONCLUSION

LH-RH analogues are effective in BBD treatment, even if they are not first choice drugs ([40]). A possible use could be hypothesized in the form of the disease that relapses and is refractory to other treatments. It could also be used in chronic cystic mastopathy in women with high risk of breast cancer in premenopause ([41, 55]).

We do not yet know much about the nature and control of growth factors which act on mammary glands. In benign breast pathology, as well as in breast cancer, this research will offer new possibilities for diagnosis and treatment.

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