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STEROID RECEPTORS IN BENIGN BREAST DISEASE, GROSS CYSTIC DISEASE AND FIBROADENOMA

G. B. NARDELLI - V. LAMAINA - F. SILIOTTI
Institute of Obstetric and Gynaecology, University of Padua (Italy)

Summary: The benign breast pathology embraces a wide variety of anatomo-clinical-pathological conditions producing confusion in nomenclature. The Authors collected three different types of BBP and investigated the hormonal receptor status for each.

The following concentrations of ERc were found: 1-6 fmol/mg in BBD; less than 2 fmol/mg in GCD; 12-18 fmol/mg in the cytoplasm and 29-37.5 fmol/mg in the nucleus in FA.

In FA, PgR was found in concentrations of 43.5-50 fmol/mg in the cytoplasm and 0.2-10 fmol/mg in the nucleus.

Even if we consider these three histo-pathological entities (BBD, GCD, FA) separately, no correlation can be seen between the presence of receptors and benign breast disease. The only observation we can make is that the fibroadenomas contain more easily identificable receptor concentrations than BBD and GCD.

Key words: receptors, benign breast disease.

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The common benign diseases of the breast are, in great part, caused by hormonal abnormalities which persist for a long time.

The hormonal stimuli change the metabolism of the cells of the mammary gland and so in the normal mammary cycle the histologic changes are present in the involutional phase. These changes (epithelial degeneration, fibrous stromal proliferation, round cell infiltration) begin one or two days before the onset of menstruation and closely resemble the more advanced changes found in fibrocystic mastopathy and fibroadenomas ([1, 2, 3, 4, 5, 6]).

The frequent association of uterine, ovarian and mammarian abnormalities forms the basis that strengthens such suppositions of a systemic cause of abnormal responses to hormones. This supports the heterogeneity of the breast tissues and, proportionally, the different levels of detectable receptors.

Many Authors have been unable to detect receptors in « BBD » specimens ([7]) while other Authors ([8, 9, 10]) observed a relationship between the presence of receptors and the degree of cellularity ([11, 12, 13]).

MATERIAL AND METHODS

29 patients were studied; 16 had BBD (Benign Breast Disease), 7 had GCD (Gross Cystic Disease) and 6 had FA (Fibroadenoma). Mammography, Thermography, Diaphanoscopy, Sonography, Cytology of the cystic liquid, fine needle biopsy, histology, colposcopy and vaginal and endometrial cytology were performed in all patients.

Fibroadenomas were surgically removed ([16, 17, 18, 19, 20, 21]).

In order to maintain the protein concentrations of the cytosol constant, only ERc were assayed in tissue fragments obtained through abundant and repeated bilateral fine needle biopsies.

Cytoplasmatic and nuclear assays of ER and PgR were performed on samples of FA. All patients were of a fertile age, 25-45 yrs., and had menstrual cycles of 25-29 days. Patients had undergone no hormonal therapy in the 6 months prior to the removal of tissue samples, which was performed in the periovulatory period (12th-14th day).

After the initial screening, all patients were treated with methylate-derived synthetic progestinic (medroxiprogesterone acetate) at 10 mg/day from the 5th to the 25th day of their cycles. Topical progesterone in a 1% concentration in hydro-alcoholic gel was also used. 5 g of gel was applied daily on both breasts from the 15th to the 25th day of the cycle, so as to obtain 5 mg of the active product. Follow-up was every three months ([22, 23]).

Chemicals and Buffers

The labelled synthetic estrogenic and progestin lingads (2.4.6H-Oestradiol-90 Ci/mmol-Amersham; Promegestone-17 Methyl-H-NET-555-NEN) and unlabelled steroids (Diethylstilbestrol-Sigma, DHT-5-Androstan-17B-ol-3-one-Merck, F-Hydrocortisone Acetate-Calbiochem, R-5020-Promegestone-Cold-NEN) were used.

The buffers used were TEDMo (TRIS 0.01M, EDTA 0.015M, DTT 0.5mM, Na_2MoO_4 5mM-12N HCl - pH 7.4/4°C), TEG-KCl (TRIS 10mM, EDTA 1mM, Glycerol 10%, KCl 0.6M, 12n HCl - pH 8.0/4 °C), TEGMoME (TRIS 50mM, EDTA 1.5mM, Glycerol 10%, MoAc 10mM, MeAc 0.4mM), TEGD (TRIS 50mM, EDTA 1.5mM, Glycerol 10%, DTT 25mM), DCC (Norit A 0.25%, Dextran Grade C 0.0025%, TRIS 0.01 M, 12-N-HCl - pH 8.0/4 °C, 1% BSA).

Cytosol and Nuclear extract preparations

The specimens were all processed for biochemical investigation less than 2 wks after delivery, and all steps were performed in Cold-Room (0-4 °C). The frozen tissue was pulverized with Politron PT 10-35 in cold-buffer (1:4 w/v).

Cytoplasmatic and nuclear fractions were separated by ultracentrifugation of the tissue homogenate in Kontron TGA-65-Rotor TFT 65.13 at 105,000 g/lh. The pellets were resuspended in TED-KCl 0.6M and sonicated with a sonic-cell-disrupter 16-850. After ultracentrifugation, the resulting supernatant was the KCl nuclear extract used for nuclear receptor assay.

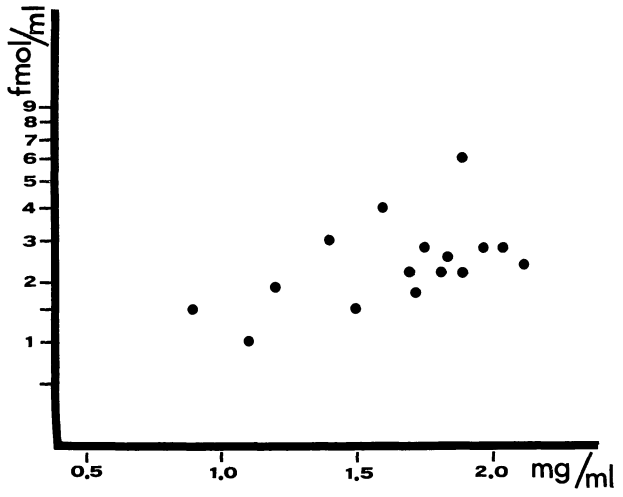
Protein Measurement

The Nucleic Acid concentrations of all specimens were estimated by the Method of E. Layne ([31]) and the protein concentrations were determined by Lowry's Method; Protein ranges of 0.75-2.0 mg/ml for ER and of 2.0-4.0 mg/ml for PgR were used ([14]).

ERc and PgRc/n binding assay

ERc and PgRC were measured by Dextran-reated exchange assay as previously described. The Kd range of ERc/n was 0.21-0.23 nM and 1.67-1.99 nM for PgRc/n ([15]).

Table 1. — *Benign Breast Diseases: the specific estrogen binding to receptor (fmol/ml) was lower in breast tissues with strong sclero-adenosis and tipical lobulo-ductal hyperplasia; therefore the specific bond increases when the protein concentrations (mg/ml) are higher. Moreover, the heterogeneicity of the tissue with proliferative and regressive alterations reduces the cellular pool receptors including.*



RESULTS

Benign Breast Disease (BBD)

At the first observation, the 16 patients with BBD showed mastodynia, micro-macronodosity and fibrosis. In these patients, tissue sample were taken twice, at the moment of the first observation and after 18 months of treatment with oral and topical progesterone, in quantities sufficient for the assaying of hormonal receptors (ER) and histology. Histology showed situations of typical lobulo-ductal hyperplasia and sclerosing adenosis. Assays for ESr were performed in the 16 patients with ascertained BBD. The quantity of tissue available and the obligation to respect the range fo protein concentrations prohibited other assays.

Table 1 shows the results obtained; the specific radio-activity for estrogen receptors oscillated between 0.9 and 2.12 mg/ml. Only a weak correlation could be seen between specific bound and higher protein concentrations. The importance of this correlation is relative since in our experience with breast tumors, levels of steroidal receptors were higher than those in the experience of other Authors ([7, 8, 9, 10, 24, 25, 26]) even though the protein concentrations were the same.

This observation can be explained by the excessive heterogeneicity of the tissue taken, and by the consequently scarce cellular component of the same. Moreover, fibrosis further accentuates cellular dispersion.

Gross Cystic Disease (GCD)

7 Patients showed a GCD ranging from 2-6 cm in diameter; Aspiration of the ([2, 6, 7]) liquid was performed from one to seven times (for a total 28 times). Liquid volume varied between 1 cc and 30 cc per patient. After aspiration, glandular tissue was taken, in ultrasonicscopy, in quantities sufficient for histology and hormonal receptor (ER) assays. Patients were then treated with oral and topical

progesterone. Varied situations of cohabitation by stroma, epithelial and apocrinal cyst in the same breast were seen cyto-histologically. The cystic disease coexisted with the dysplasia in its various manifestations of ductal and lobular hyperplasia, adenosis and fibrosis. Cystic liquid and serum were taken contemporaneously and assays were performed on both for the parameters reported in table 2.

Table 2. — *No correlations have been found among follicle stimulating hormone, luteinizing hormone, estradiol, progesterone, prolactin, testosterone levels in cystic fluid and serum.*

		Cystic fluid	Blood
FSH	mU/ml	3.5 - 25	5 - 30
LH	mU/ml	5 - 100	10 - 120
E_2	pg/ml	115 - 195	200 - 250
E_3 t.	pg/ml	0.7 - 20	—
Pg	ng/ml	1.9 - 29.7	3 - 35
PRL	ng/ml	2.9 - 25.5	3.5 - 29.7
T	ng/ml	0.1 - 0.5	0.1 - 1.5

The concentrations of prolactin, LH, FSH, in cystic fluid are similar to those found in blood, although progesterone seems to be lower; In contrast, steroids such as progesterone and testosterone are present in similar concentrations to those found in cystic fluid in variable amounts. The amounts for cytoplasmatic estrogen receptors were classified as negative, with levels of less than 2 fmol/mg in all cases.

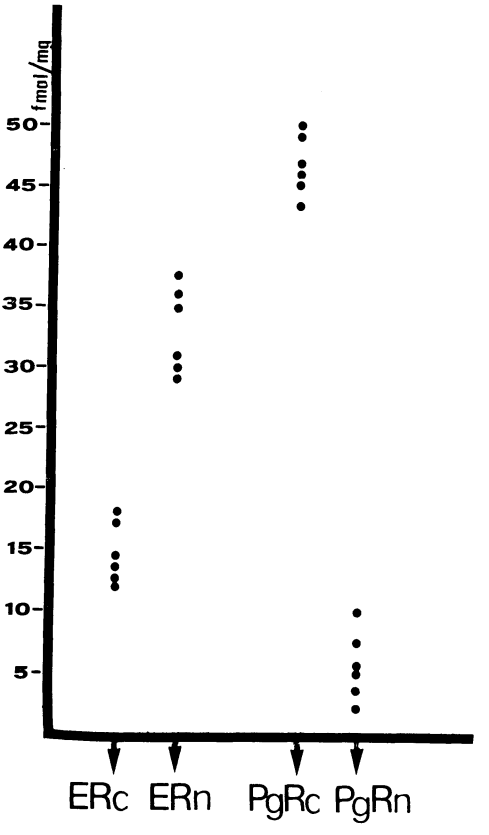
Fibroadenomas (FA)

6 Patients had solitary solid formations ranging from 1.5 to 4.0 cm in diameter. These formations were surgically removed and histology and hormonal receptor assays (ERc/n, PgRc/n) were performed on each one. Patients were then treated with oral or topical progesterone. Histology confermed the existence of fibroadenomatoid mastopathy and focal fibrosis. The fibrosis grading estimated microscopically was less than 25% of the cellular population present in our samples ([1, 10, 12, 13, 21, 25]).

Cytosolic and nuclear estrogen receptors (ERc/n) and progesterone receptors (PgRc/n) were assayed in 6 specimens removed at 12th - 14th days of the cycle. Estrogen receptors ranged between 12-18 fmol/mg in the cytoplasm, and between 29.0-37.5 fmol/mg in the nucleus.

The progesterone receptors ranged between 43.5-50.0 fmol/mg in the cytoplasm and between 0.2-10.0 fmol/mg in the nucleus (tab. 3).

Table 3. — *Fibroadenomas: the tissue taken in periovulatory phase (12th-14th day), showed low levels of receptors but higher ER concentrations in the nucleus than in the cytoplasm, in contrast with higher PgR concentrations in the cytoplasm than in the nucleus.*



The ER were more concentrated in the nucleus while the PgR were higher in the cytoplasm, according to ovulation phase.

DISCUSSION

The tissues which characterize all benign breast diseases present a wide cellular heterogeneity (epithelial, stromal, and adipose cells) and reflect the functional organization of the breast itself ([6, 7, 11, 27]).

Fibrous tissue and Cooper's ligaments with their tri-dimensional architecture are also present. Therefore, it is easy to see how a biopsy sample can furnish the laboratory with a « cellular pool » with unpredictable low, medium or high percentages of epithelial cells.

Even though fibroadenomas are considered a benign breast disease with a homogeneous epithelial concentration, they sometimes present an exclusive proliferation of the acinose epithelial cells without fibrosis and at other times present a fibrosis that is so exalted as to hide the original lobular proliferation.

They may also present all of the intermediate situations of cell fibrosis co-habitation. This explains the World Health Organization's definition of mastopathy as a spectre of proliferative and regressive alterations of the breast tissue with an abnormal presence of epithelial and connective elements which co-exist in unpredictable percentages.

On the other hand, the contemporaneous presence of epithelial and connective elements with different grades of alteration (apocrine metaplasia, typical and hyperplasia etc.) accounts for the different nucleo-cytoplasmatic characteristics which consitute a theoretically homogeneous cellular pool in which to verify receptorial kinetics.

In evaluating such variability, the day of the cycle must be taken into account as well. Therefore, we are convinced than the study of intracellular receptors offers no indications for an aimed therapeutic support in benign breast diseases.

It must also be kept in mind that hormonal receptors do not inform us as to the potential state of malignancy of what is belived to be benign tissue. Therefore, the use of these receptors has yet to be verified in this specific sectors. The use of the receptors will probably be re-evaluated when we can study them in pre-selected cellular populations with monoclonal anti-antigen membrane antibodies (Monoclonal antobodies against milk-fat globule or breast epithelial cell line) ([28, 29, 30]).

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COMBINED PELVIC SONOGRAPHY AND SERUM BETA hCG, VERSUS LAPAROSCOPY FOR THE DIAGNOSIS OF STABLE PATIENT SUSPECTED OF ECTOPIC PREGNANCY

R. ACHIRON - E. SCHEJTER - H. ZAKUT

Department of Obstetrics and Gynecology,
The Sackler Faculty of Medicine, Tel-Aviv University,
The Edith Wolfson Medical Center, Holon 58100 (Israel)

Summary: The role of sonography in stable patients suspected of ectopic pregnancy is to establish the diagnosis using positive, suggestive or negative signs.

Establishing whether or not intrauterine gestation is present is crucial, as is the detection of any extrauterine abnormality. Sonography may be normal in ectopic pregnancy or when it is not abnormal findings are frequently nonspecific. Therefore, the sonographic results must be correlated and integrated with the clinical history and findings as well as with other diagnostic procedures.

The combination of ultrasound scannning with beta hCG was found highly contributory to the determination of the existence of an ectopic pregnancy.

Understanding the objectives and limitations of each diagnostic test involved is essential for logical and optimal sequences of diagnostic procedures to be employed in patient management.

During a twenty-month period, 138 patients were examined due to clinical suspicion of "subacute" ectopic pregnancy. Sixty-one patients were managed according to a non-invasive protocol composed of: a) ultrasound scanning alone and b) ultrasound scanning combined with serum beta subunit hCG.

Ultrasonograms for ectopic pregnancy diagnosis were coded: positive (fluid in cul-de-sac or extrauterine sac); suggestive empty uterus, adnexal mass and pseudo-gestational sac) and negative (intrauterine gestational sac and normal pelvis).