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Biology of Breast Cancer: Receptors; Workshop Report

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INTRODUCTION

THIS poster session and the ensuing workshop were devoted to the endocrine function in patients with breast cancer (BC), the mechanisms of action of hormones and antihormones at the tissue level and various problems related to steroid receptors. This represents a broad spectrum of studies adding pieces of information to an important field of BC research. None purports to settle a given question but rather to update current knowledge. In order to avoid presenting a lengthy and heterogeneous catalogue, this report will consider only six topics, about which a significant number of posters were displayed. Only those posters will be analysed here. The authors whose contributions are omitted will hopefully forgive us and are assured that no other consideration was involved in the selection.

ENDOCRINE FUNCTION AND BREAST CANCER

Read *et al.* (Cardiff, U.K.) [1] used very sensitive RIAs to measure progesterone and estradiol in the saliva. The collection of saliva samples, being non-invasive, is well suited to aetiological studies. The progesterone assay provides information on the proportion of anovulatory cycles which occur in adolescent girls, a subject relevant to the increased risk of BC associated with early menarche. The oestradiol assay, which provides a means of assessing plasma free oestradiol levels, has been measured in postmenopausal women with BC and in controls.

Hawkins *et al.* (Edinburgh, U.K.) [2] measured oestrone sulphate in cow's milk and cream (low levels), in plasma from postmenopausal women (higher levels in BC cases) and in cystic fluid from premenopausal women (high levels). They also measured the capacity of various tissues for cleavage of [^{3}H]-oestrone sulphate. They concluded that (1) the dietary consumption of oestrone sulphate in milk/cream is insignificant; (2) breast cysts contain high concentrations of the hormone; and (3) breast tissues, especially malignant and adipose tissues, contain oestrone sulphatase activity, the significance of which is uncertain.

Chetty *et al.* (Edinburgh, U.K.) [3] looked for an abnormality of the adrenal response to ACTH in primary BC patients. The number of patients was quite small (17 patients; 18 controls), so that after appropriate stratification firm conclusions can hardly be drawn. It was found that in postmenopausal women $\beta^{1\text{-}24}$ACTH produced an abnormally high response of DHA and cortisol but not of DHAS, while in premenopausal patients there was no such abnormality. In this small study stress may have played a role as well as other biases, and much caution should be used in the interpretation of the results.

MECHANISM OF ACTION OF HORMONES AND ANTIHORMONES

The first poster under this heading was by Raam and Richardson (Boston, MA, U.S.A.) [4], who studied the cytoplasm–nucleus translocation of oestrogen receptors (ER) in various tissues *in vitro* under the effect of oestrogens and anti-oestrogens. For that purpose they used a polyclonal antibody which they claim is specific for ER and can be utilized to locate ER in cell or tissue preparations by an immunofluorescent (IF) method reported earlier [5]. When oestrogen-starved normal human endometrial cells in short-term culture are exposed for 2 hr in the presence of oestradiol (E_2), diethylstilboestrol (DES) or hydroxytamoxifen (OH-TX), complete transloca-

tion of the IF occurred in all cells. There was one endometrial carcinoma in which translocation did not occur. When MCF-7 cells were grown in the continuous presence of oestradiol the IF was exclusively intranuclear. When the same cells were maintained for 5 days in an oestrogen-free medium the whole IF became located in the cytoplasm. Then, after 2 hr exposure at 37°C to E_2, DES or OH-TX, total nuclear translocation of IF occurred in 50% of the cells but was delayed in 40% and absent in 10%. The results of longer exposure times was not reported. The authors' conclusion was that anti-ER antibodies proved to be an excellent test for studying abnormalities of ER translocation and that it should be applied to biopsies of human BC to investigate the problem of the refractoriness to endocrine therapy of some 40% of the ER+ cases (ER defectivity?).

The poster by Taylor *et al.* (Bern, Switzerland) [6] addressed the important problem of the antioestrogen (AE) binding component and its putative role in the growth control of BC. They found that two ER+ cell lines as well as one ER- line take up 20–100 times greater amounts of radiolabelled tamoxifen or hydroxytamoxifen than of radiolabelled oestradiol, both in the cytoplasm and nucleus. In the cytoplasm uptake of AE is non-saturable. In ER+ cell lines the nuclear uptake of AE is saturable and is associated with the presence of a high-affinity binding site of AE which may be competed for by AE but not by oestradiol. In the ER- cell line the nuclear uptake of AE is as large as in ER+ cells, but these cells do not contain the high-affinity binding component of AE. The authors are presently studying the correlation between the presence of AE binding component and the growth-inhibitory effect of these compounds.

Sato *et al.* (Osaka, Japan) [7] reported agonist and antagonist effects of oestradiol in the rat uterus as revealed by the analysis of mRNAs after their purification and cell-free translation with rabbit reticulocyte lysate and [^{35}S]-methionine. The administration of oestradiol, diethylstilboestrol or tamoxifen to ovariectomized rats resulted in the appearance of four new mRNAs in the uterine tissue and their disappearance after hormone withdrawal. If huge doses of oestradiol were repeatedly administered at 24-hr intervals, maximum induction of the four mRNAs was observed after 3 successive doses, but they completely disappeared after a 7-day treatment without alteration of the other mRNA pattern. These results are interpreted to indicate that oestrogens have agonist or antagonist effects on uterine mRNA induction depending upon duration of administration of huge supra-physiologic doses.

RECEPTORS METHODOLOGY

Measurement of oestrogen receptors (ER) and progesterone receptors (PgR) is now carried out in most laboratories by the dextran-coated charcoal assay (DCC). The method is convenient, reliable and reproducible. It has disadvantages such as being rather time consuming, not readily automated and requiring a substantial amount of tumor tissue. Attempts are therefore made to circumvent these problems. They mainly involve the isoelectric focusing technique, which allows work on smaller samples, high-performance liquid chromatography, which combines the same advantage with that of being faster and automatable, and finally various histological methods. The latter are using either fluorescent oestrogen derivatives which have been claimed to bind to the ER, antibodies against oestrogens which presumably are present in ER-rich tissues or antibodies against the ER itself. The posters which were displayed in this section are examples of one or another of these attempts.

Two posters were dealing with histochemical or immunohistological methods of 'ER assessment' in breast cancer. Vroom *et al.* (Amsterdam, The Netherlands) [8] used the fluorescent probe E_2-BSA-FITC on sections of 37 consecutive samples of primary BC and compared the results with those of the classical DCC biochemical assay. Although a statistically significant correlation was observed, this small series does not lend itself to a straightforward interpretation. Iwasa *et al.* (Osaka, Japan) [9] studied breast tissue fixed by the freeze-substitution method and 'assessed ER' with anti-17β-oestradiol-6-BSA, although it was not clear how they validated their method. It is our impression that these two posters add little to an already confusing literature.

Another poster by De Goeij *et al.* (Maastricht, The Netherlands) [10] featured "a new quantitative ER assay on frozen sections using radiolabeled estradiol". As claimed by the authors, the method lends itself both to Scatchard plot analysis and autoradiography. Evidence is presented for specificity of the method when applied to normal tissues but no data are provided to correlate the results with those obtained with classical methods. It is felt that more work is needed before this technique can be applied for research or for clinical purposes.

Peterse *et al.* (Amsterdam, The Netherlands) [11] reported on the use of the isoelectric focusing technique [12] as applied to fine-needle aspiration biopsy. They first compared this micromethod with the classical dextran-coated charcoal assay on tissue specimens from 29 cases of primary or recurrent breast cancers. The correlation was very

significant ($P < 0.001$) and especially good with aspirates that were rich in cells. So far 193 fine-needle aspiration biopsies have been studied. The results were considered reliable in 166 cases (ER+, 42 cases; ER±, 21 cases; ER–, 103 cases) and unreliable in 27 (poor cellularity in 14, too much blood in 8 and technical failure in 5). Recent data suggest that the use of the synthetic ligand [^{3}H]-R2858 might improve the results since it eliminates the interference with serum proteins. Our personal belief is that this elegant method, which requires special equipment, is difficult to use and that its reliability remains to be firmly established. Its application to multi-institutional trials is not yet at hand and its use restricted to especially skilled laboratories, and it is still of an experimental nature.

The last paper in this section was presented by Jawny *et al.* (München, F.R.G.) [13]. It recommends the use of 'saturation analysis', a new way to calculate kinetic parameters of ER assay according to the mass action law, instead of other methods, including the Scatchard plot. Evaluation of the theoretical and practical aspects of this method would require a close scrutiny to a full-length paper and further discussion.

RECEPTORS ANALYSIS: QUALITY CONTROL AND STANDARDIZATION

Standardization of ER assay has long been a major preoccupation within the EORTC Breast Cancer Cooperative Group and a standardized method has been published [14] and later revised [15]. More recently, the importance of using the receptors values in multi-institutional clinical trials, especially in the adjuvant setting, was recognized and an interlaboratory quality control schema was established in 1979. The results of this major work were reported by Koenders and Thorpe (Nijmegen, The Netherlands; Copenhagen, Denmark) on behalf of the EORTC Receptor Group [16]. Lyophilized samples were chosen as a reference preparation because of their thermostability. Trials were conducted with this material in 1981 and 1982. Samples were sent to 13 laboratories in 7 countries for assessing intra- and interlaboratory consistency. During both trials interlaboratory variation was smaller than in pilot studies carried out in previous years. Recalculation of the raw data of all participants by a common computer program further reduced this variation. In addition to the quantitative studies described above, the degree of agreement between labs on the classification of samples being receptor-positive or -negative was investigated. Samples with high receptor content yielded a 100% agreement while samples with low receptor content yielded false-negative results in 5% and 14% respectively for ER and PgR assays. Overall the PgR assay gave much less consistent results and a special effort is needed for reaching better uniformity in this area. Finally, a standardization program for laboratories participating in multicenter clinical trials, guided by receptor assays, is being set up. In the opinion of the rapporteurs, this work was the most important of the workshop not only because of its quality but also because of its impact on clinical aspects of the EORTC Breast Group activities.

Another poster on the same subject was displayed by Agrimonti *et al.* (Torino, Italy) [17]. It deals with a study involving 24 Italian laboratories and bears especially on the evaluation of the various methods available for the calculation by the computer center of the raw data provided by the laboratories. This study, like the previous one, stresses the importance of a uniform method of computation of the experimental data.

RECEPTORS AND PROGNOSIS

The presence of ER in primary BC has been shown to be of favourable prognosis, independently of the axillary nodal status [18]. This observation has generally been confirmed [19], although not universally [20]. In his plenary Wassink Lecture at the outset of this Conference, McGuire (San Antonio, TX, U.S.A.) [21] reported his analysis of the prognostic importance of ER and PgR in a randomized clinical trial of adjuvant therapy for stage II breast cancer. Both parameters were significant predictors of prolonged disease-free survival when analysed separately. However, when ER and PgR were analysed together in multivariate models, ER was never a significant predictor while PgR and number of positive nodes always remained significant prognostic factors. In another large series of patients with primary BC subjected to modified radical mastectomy, the analysis of the various prognostic factors by the Cox model disclosed that ER was of no prognostic value whatsoever when the histological grading of malignancy was included [22]. This section featured posters dealing with this controversial subject.

Heise and Görlich (Berlin-Buch, G.D.R.) [23] studied the influence of ER on the fate of patients with primary BC. They first observed that the ER level and incidence were much dependent on the phase of the menstrual cycle, being higher in the early proliferative phase and lower in the early secretory phase. Besides, they found that the disease-free and total survivals were longer in ER+ than in ER– patients. It should be stressed,

however, that the authors did not take into consideration such important prognostic factors as nodal status and histological grading of malignancy.

Eiermann *et al.* (München, F.R.G.) [24] analysed 1500 BC specimens for ER and PgR content at the time of primary surgical therapy. Of these, they selected 201 patients for whom valid information (histology, tumor grade, clinical follow-up) was available and duration of observation long enough (30 months). They concluded that the relapse-free survival at 30 months was longer in ER+PgR+ than in ER-PgR- patients and that the prognosis was intermediate when only one receptor was positive. This study should be interpreted with great caution in view of the strong possibility of a selection bias.

Berlie (Saint-Cloud, France) [25] presented a collaborative study of five French anticancer centres. They analysed a very special group of patients with grade III tumors, according to Bloom and Richardson, and absence of nodal involvement (N-). In this series there was no difference in disease-free or total survival according to the PgR status. It should be stressed, however, that the duration of follow-up was very short (median, 2 yr), that the nature of the treatment was not specified and that there was no quality control study for the PgR assay among the five centres.

Passalacqua *et al.* (Parma, Italy) [26] analysed a series of 214 patients of whom 41 had advanced disease at the time of diagnosis. The median duration of follow-up was 25 months. The distribution of these patients according to receptor status was: ER+PgR+, 110; ER+PgR-, 71; ER-PgR+, 2; ER-PgR-, 31. In the 173 patients with operable disease the N+ and N- were equally distributed in the receptor categories. In these patients the relapse-free survival was significantly longer in the ER+ PgR+ than in the ER+PgR- cases but the total survival was not. ER-PgR- cases had the worst prognosis.

Campbell *et al.* (Nottingham and Cardiff, U.K.) [27] presented a provocative poster claiming that quantitative ER measurement obviates the need for PgR analysis in primary BC. PgR and ER were assayed in 167 primary BC. Patients were followed without adjuvant therapy for a median interval of 3 yr. There was a distinct interrelationship between ER and PgR: the higher the ER concentration of a cancer the more it was likely to be PgR-positive. With regard to prognosis, PgR status was unrelated to disease-free interval or survival. Where both receptors were used in combination the prognostic power of ER was not improved. Quantitative ER defined three prognostic categories according to receptor concentration: the higher the ER concentration the better the survival ($P < 0.005$; log-rank). The authors' conclusion is that quantitative ER assays outweigh the predictive value of PgR assays in prediction of response* and prognosis.

It is our opinion that whereas the first three papers in this section are of difficult interpretation, the last two would assign a distinct prognostic value to the receptors, although it is not clear whether PgR and ER produce a different type of information. It should, however, be noted that the series are small and the duration of follow-up rather short. Moreover, it would be of interest to assess the prognostic value of steroid receptors in comparison to that of the histological grading of malignancy, a factor known for years to be of strong prognostic significance [28].

HISTOLOGY AND RECEPTORS

The poster by Contesso *et al.* (Villejuif, France) [28] presents an extensive study of the relationships between ER and PgR on the one hand and histological features of BC on the other hand. It is based on an analysis of a large series of 511 operable adenocarcinomas. The results are as follows. There was no correlation between receptors and tumor size. Among the infiltrating ductal carcinomas, the highly differentiated ones were more often ER+ PgR+ (69%) than the anaplastic ones (33%), both in pre- and postmenopausal women. Special types, except for the infiltrating lobular carcinoma, were largely devoid of receptors. There was a highly significant correlation between histological grading and rate of receptor positivity: 57% of the grade I tumors were ER+PgR+ as opposed to 25% of the grade III tumors ($P < 0.001$). Inflammatory reaction was more often reported in ER-PgR- tumors ($P = 0.002$), while elastosis was positively correlated with the presence of the two receptors. Finally, ER+PgR+ cases were equally distributed in N+ and N- cases. It should be noted that the authors did not analyse the ER+PgR- cases.

*Not summarized here.

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