Quality Control Requirements in Estrogen Receptor Determination

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Abstract

The clinical significance of estrogen receptor (ER) levels determined in breast cancer tissue depends on the quality of the ER assay procedure itself as well as on several critical factors beyond the direct responsibility of the receptor laboratory. A novel immunobiochemical assay (ER-EIA) has been made available by Abbott Laboratories. In comparison to the classical radioligand binding assay, the use of ER-EIA resulted in lower intra- and interassay as well as interlaboratory coefficients of variation. This may be explained by the complete standardization of ER-EIA as compared to ER radioligand binding assay. Considerable attention must be paid to the source of origin of quality control material if identical ER levels are to be obtained by both methods, as the monoclonal antibodies of ER-EIA bind less avidly to cytosolic ER of calf uterus and, in contrast to routine ER radioligand binding assay, are able to recognize occupied ER.

Introduction

It is well known that the classical radioligand binding assay presently used for routine steroid hormone receptor measurement in breast cancer is a complex biochemical procedure, beset with technical difficulties and methodological variations (1). Consequently various groups have taken the initiative to develop national and/or supranational programs for quality assurance of RBA2 (2, 3).

Recently, specific monoclonal antibodies to the estrogen receptor protein have been prepared (4) which can be used for biochemical (5) and cytochemical (6) ER determination. Enzyme immunoassays based on monoclonal antibodies to ER have become available, both for the biochemical (ER-EIA) and cytochemical (ER-ICA) analysis of the receptor protein.

The quality control requirements for biochemical estrogen receptor determination by classical and novel methodologies are outlined in this paper. It is stressed that the clinical usefulness of ER levels in breast cancer tissue does not solely depend on the quality of the assay procedure itself, but on several other critical factors outside the direct responsibility of the receptor laboratory.

Critical Factors Influencing ER Content in Vivo

The influence of endogenous and exogenous hormones and antihormones on ER levels must be taken into consideration. High plasma levels of estrogens and the antiestrogen tamoxifen translocate ER to the nucleus, thereby reducing ER number in low salt tumor cytosol, and may cause false-negative results in RBA and ER-EIA. Therefore, in premenopausal patients, ER analyses from tissues taken in the early proliferative phase of the menstrual cycle are more reliable than those from tissues obtained in the luteal phase.

The difficulties can be easily overcome by applying ER-EIA to nuclear extracts and/or ER-ICA to fixed, frozen tissue sections. In tissue samples taken during or immediately after radiotherapy or chemotherapy, a decrease of ER concentration and a decrease in the percentage of ER positive specimens may be observed (7, 8).

Logistics of Tissue Handling

To obtain the proper tissue sample for receptor analyses, the cooperation of surgeon, pathologist, and receptor laboratory is of paramount importance. Independent of the method applied for receptor determination (RBA, ER-EIA, ER-ICA), tissue processing by surgeon and pathologist should be identical. Receptors measured by RBA are thermostable. As long as the stability of receptors determined by means of immunological technique is not clearly proven to be superior to that measured by classical RBA, the same precautionary measures have to be taken to avoid loss of receptors in the tumor tissues. In this context, the following recommendations are made (9–11): (a) At the operation theater the tissue sample has to be cooled immediately to ice temperature and kept cold while being transported to the pathologist. (b) At the pathology laboratory the sample is freed from surrounding nonmalignant tissue and a piece of the tissue sample directly adjacent to the piece for receptor determination should be histologically examined to ensure that the sample to be sent to the receptor laboratory is tumor tissue. Any contact with fixing agents (e.g., formalin) must be avoided. (c) For shipment to the receptor laboratory the tissue should be kept on dry ice.

Quality Control of Transportation System

Recently, our laboratory has developed tablets containing defined concentrations of steroid hormone receptors.3 The tablets can be used for quality control of the transportation system. Primarily, the receptors in the tablets are thermostable. On removal of the tumor tissue in the operating theater the tablets can be easily activated. At that very moment, the receptors in the tablets become thermostable as are the receptors in the tumor tissue. The activated tablet is then transported together with the tissue sample. The degree of preservation of the receptor concentrations in the tablets can be used as a measure of the quality of the transportation system.

Quality Assurance of Classical RBA

The RBA method on which to date most of the national and international study groups have agreed is the multiple point dextran-coated charcoal assay analyzed by Scatchard plot (12). The various reported national and supranational quality control programs have disclosed certain problems of this classical assay (1–3, 13, 14). From these studies and the work of others the following steps of the assay have emerged as being especially critical and prone to errors: homogenization procedure (10, 11); composition of the homogenization buffer (10); selection of labeled and unlabeled steroids; cytosol protein concentration (2, 15); dextran-coated charcoal composition; final concentration and contact time with labeled cytosol (2); protein determination (2); estimation of counter efficiency; and calculation method (14).
QUALITY CONTROL OF ER DETERMINATION

Comparison of ER-EIA and ER-RBA

Using lyophilized human breast cancer cytosol as quality control samples, we recorded the intra- and interassay variations of the Abbott ER-EIA in comparison to those of the conventional multiple point dextran-coated charcoal method performed according to the standard European Organisation for the Research and Treatment of Cancer procedure (11). For ER-EIA the intraassay coefficient of variation was 2.2% as compared to 4.9% for ER-RBA. The interassay CV was found to be 7% for ER-EIA and 9.2% for ER-RBA.

Subsequently, interlaboratory variations in ER measurement by ER-EIA and ER-RBA were studied using lyophilized calf uterus cytosols prepared at the author's institution.

As shown in Table 1, depending on the receptor concentration in the lyophilized samples, interlaboratory CV for ER-RBA ranged from 15 to 21%. When the same samples were analyzed by ER-EIA, the interlaboratory CVs were found to be independent of ER concentration and lower, ranging from 10 to 13%. As can also be seen, receptor concentrations determined by both methods differed remarkably. However, for all samples analyzed the ER-RBA/ER-EIA ratio remained constant (2.6).

In striking contrast, in the lyophilized human breast cancer cytosols as well as in human breast cancer tissue (16), there were no differences in the receptor levels determined by ER-EIA as compared to ER-RBA. However, similar to our observation, ER concentrations determined by both methods differed when lyophilized cytosols from calf uterus (ER-RBA/ER-EIA ratio, 5; different source of material) or MCF-7 cells, grown in the presence of estrogens (ratio, 0.4) were used.

Obviously, the monoclonal antibodies which were raised against cytosolic human breast cancer ER from MCF-7 cells bind less avidly to ER from calf uterus. The low ER-RBA/ER-EIA ratio found in lyophilized MCF-7 cytosols may be due to the presence of occupied ER which is not detected by routine ER-RBA.

Concluding Remarks

The clinical significance of receptor determination in breast cancer critically depends on the quality of the receptor assay procedure itself as well as on several factors beyond the direct responsibility of the receptor laboratory. Therefore, standardization of the method and the establishment of efficient intra- and interlaboratory quality control as well as supervision in and check of tissue handling in the hospitals and quality control of the transportation system of the tissues to the receptor laboratory are essential. Presumably due to the complete standardization of the ER-EIA assay, the intra- and interassay as well as the interlaboratory variations proved to be superior with ER-EIA as compared to ER-RBA.

As it is easy to foresee that the novel methodologies for receptor determination will be widely used in the very near future, they have to be urgently included in the various programs for quality assurance. In this context it is stressed that the source of origin for the preparation of quality control materials merits considerable attention to render the receptor concentrations determined by ER-EIA and ER-RBA quantitatively comparable. In order to preserve and improve the predictive value of biochemical ER assays the novel immunohistochemical assay should be introduced in experienced laboratories which not only guarantee a high standard of assay performance but also have organized an efficient transportation system and, by cooperation with surgeons and pathologists, ensure an adequate handling of the breast cancer tissue which is of paramount importance for the reliability of the receptor status.

References

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Cancer Res 1986;46:4249s-4250s.

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