Abstract

A new enzyme immunoassay (Abbott ER-EIA Monoclonal) for the determination of estrogen receptor in cytosols from breast tumor specimens has been developed by Abbott Laboratories. To establish the correlation of the results from this new technique with currently existing steroid binding methods, a multicenter study was conducted in eight European laboratories. All participants followed the same protocol consisting of a familiarization phase, a proficiency evaluation, and a comparison of existing steroid binding methods with the new immunoassay using panel samples and clinical specimens. ER-EIA was compared with the multipoint dextran coated charcoal assay in six laboratories, four of which followed the EORTC protocol; of the remaining two laboratories, one used a single saturating dose assay, the other an isoelectric focusing assay. The results show no significant difference between reducing agents when used in the ER-EIA. Reproducibility for the immunoassay (interassay coefficient of variation, 6%; interlaboratory coefficient of variation, 11–19%) was somewhat better than that for the steroid binding methods (interlaboratory coefficient of variation, 12–32%). The correlation between the methods was dependent on the origin of the lyophilized specimens. In breast tumor samples, an excellent correlation, (not statistically different from 1) was found between the ER-EIA and the steroid binding method in six laboratories. One laboratory showed a slope of 1.1 for the correlation line; the laboratory using isoelectric focusing showed a slope of 1.9. The mean value determined by the enzyme immunoassay in premenopausal women was 74 fmol/mg cytosol protein, and in postmenopausal women it was 187 fmol/mg cytosol protein with no significant difference in the slope of the correlation line. Results suggest the usefulness of the new standardized enzyme immunoassay for routine use in the clinical laboratory.

Introduction

In the management of breast cancer the determination of ER levels in cytosols from tumor specimens helps in the establishment of therapy schemes and has a predictive value for the course of the disease (1, 2). Several assays have been developed for the measurement of ER in human breast cancer specimens (3). All of them are based on the evaluation of the binding capacity of tumor cytosols for radiolabeled estrogens and therefore cannot reliably measure receptors in the presence of high concentrations of endogenous estrogens or antiestrogens. This difficulty has recently been overcome by the introduction by Abbott Laboratories of a new assay system that is independent of steroid binding. These assays, which are based on monoclonal antibodies to human tumor ER produced by Greene et al. (4), use the direct antigenic recognition of the receptor molecules. The antibodies recognize the ER independent of the presence or absence of estradiol in the binding site. The new assay is a solid phase enzyme immunoassay (ER-EIA) based on the “sandwich” principle as described in “Materials and Methods.”

A multicenter trial was conducted in Europe under the auspices of Abbott Laboratories to evaluate the accuracy of the ER-EIA kit. Eight laboratories routinely engaged in performing receptor measurements (DCC assays in 7 laboratories; isoelectricfocusing in one laboratory) participated in this trial. They had the following 2 study objectives: (a) To determine the reproducibility of the ER-EIA. (Interference of reducing agents commonly used in ER assays (5, 6) was additionally examined in some laboratories. All of these investigations were performed on lyophilized cytosols in which ER contents were unknown by all investigators.) (b) To compare ER levels in a series of locally prepared human breast cancer cytosols as determined by the ER-EIA and the ER assay currently in use in the participating laboratory. Details and results of this study are reported in the present paper.

MATERIALS AND METHODS

Principle of the Abbott Receptor Assay

In this assay, beads coated with one monoclonal anti-ER antibody are incubated with the tumor cytosol or appropriate standards containing ER concentrations to cover the range from 0 to 500 fmol/ml (lyophilized material is solubilized at the time of the experiment). During incubation, ERs bind to the beads and unbound material is then removed by aspiration and washing.

In the next step, a second monoclonal anti-ER antibody conjugated with horseradish peroxidase is incubated with the beads to measure the amount of bound ER. After aspiration of the excess conjugate and washing, the beads are incubated with hydrogen peroxide and o-phenylenediamine. The intensity of the color developed under this condition, which is proportional to the amount of ER in the sample, is read with a spectrophotometer at 492 nm. A straight line is obtained by plotting the absorbance of the standards versus their ER concentrations; receptor concentrations of the tumor cytosol can easily be determined from this standard curve. In each experiment, an additional control (lyophilized cytosol) was run as a check on the assay performance.

Study Protocol

Familiarization Testing of ER-EIA. In a preliminary phase, each investigator was provided with ER-EIA kits and lyophilized test specimens containing various levels of ER. Each day's assay was performed in replicates of 5. Two different proficiency panels were used by 2 independent groups of investigators.

Comparison of the ER-EIA with the Investigator's Routine Binding Assay. Over a 3-day period, each investigator tested 3 lyophilized specimens containing different concentrations of ER. Samples were again tested in replicates of 5. In addition, some investigators evaluated the effect of reducing agents on ER-EIA. The influence of MTG or
Clinical Specimen Testing

Cytosols from primary or metastatic breast cancer tissues were assayed by both ER-EIA and the investigator's routine binding assay. Specimens for ER-EIA were analyzed at 2 dilutions in the investigator's routinely used homogenization buffer. The first dilution was prepared by adjusting the protein concentration to 1–2 mg/ml, the second dilution by diluting the adjusted sample 3-fold in the same buffer. Binding assays were performed without any special dilution. For every sample evaluated, extensive patient clinical history information was required before testing.

Preparation of Cytosols

Lyophilized Samples. Cytosols were reconstituted before assay by adding 5 ml of ice-chilled 10% (v/v) aqueous glycerol solution containing either MTG or DTT (see above). After complete dissolution of the powder, the cytosols were immediately assayed.

Tumor Specimens. The investigator's routine procedures were used to prepare tumor cytosols (7).

Receptor Assays

ER-EIA. Assays were run according to the manufacturers' instructions.

Binding Assays. Multipoint DCC assays (3) were run in 6 laboratories, 4 of them according to the standard European Organization for the Research and Treatment of Cancer procedure (7, 8). Data were analyzed according to Scatchard (9). Single point DCC (3) or IEF (3, 10) assays were performed in 2 laboratories.

Protein Assays. Protein concentrations were determined by the investigator's routine procedure [Lowry et al. (11) or Bradford (12)]. An Abbott suggested protein assay (modified Lowry) was run in parallel as an additional control. ER concentrations were expressed according to routine procedure.

Analysis of the Data

All data were recorded on special forms and sent to Abbott for final review by G. L.
Abbott (modified Lowry) yielded comparable protein concentrations (data not shown). Statistical analysis of the data was performed according to the method of passing and Bablock (15, 16). This procedure allows the comparison of 2 assay methods in which both variables are prone to error. Linear regression could not be used since none of the variables could be assumed to be free of error.

For all investigators using the DCC method except one, a linear correlation between their routine methods and ER-EIA, not significantly different from 1.0 (within 95% confidence interval) could be found (Laboratory B slope, 1.1). With the exception of Laboratory B (i = 2) and Laboratory F (i = 5), intercepts were found not to be statistically different from 0 (Table 1).

Analysis of pooled data from pre- and postmenopausal patients (Fig. 4) revealed differences in the mean receptor level expressed in fmol/mg protein (ER-EIA values: premenopausal, 74; postmenopausal, 187). The slopes of the correlation lines were not significantly different (premenopausal, 1.2; postmenopausal, 1.1). Correlation with further clinical data will be published elsewhere.

Discussion

These results demonstrate the usefulness of the ER-EIA kit for the measurement of ER in mammary tumor cytosols. Studies on lyophilized samples established the excellent reproducibility of the assay. Inter- and intralaboratory variation coefficients were lower than those reported in most quality control laboratories using DCC assays (13, 14). This is most probably due to the total standardization among laboratories of ER-EIA which cannot be achieved with DCC assays. Multiple sources of variability have been identified in the latter; i.e., type of labeled ligands, ratio of cytosol to incubation volume and/or total volume during DCC extraction procedure, composition, and contact time with DCC (13). Interpretation of the binding data (Scatchard plot analysis) is another source of error (17). Furthermore, multipoint DCC assays require a minimal cytosol volume which cannot be easily obtained with small tumor lesions (approximately 10 x 100 ^1 cytosol at 1 mg/ml). The smaller amount of cytosol (2 x 100 ^1 cytosol at 1-2 mg/ml) required by the ER-EIA makes it appropriate for all sizes of tumor samples, even microparticles obtained by drill biopsies or fine needle aspirates (18). IEF has been reported to be another method of choice for the receptor assessment in such microtumor samples (fine needle aspirates) (19). This method, unfortunately, requires equipment not available in all laboratories and, moreover, is more time consuming than ER-EIA. Thus, all these data confer a significant practical advantage to ER-EIA over present routine assays.

Breast cancers are characterized by a wide range of ER concentrations (10 to 1000 fmol/mg protein) (20). This observation led to the concept that quantitative assessment of ER is required for the characterization of the hormone sensitivity of mammary tumors. The hypothesis was confirmed by the demonstration that, in the advanced stage, the probability of obtaining a remission with endocrine therapy is related to the ER content of the tumor (1, 21-25). The data reported here on a large series of breast cancer specimens (total, 557) established...
the quantitative relevance of ER-EIA. Statistical analysis of the data demonstrated the excellent correlation between both assays. Moreover, a correlation line with a slope of approximately 1.0 and a Y-axis intercept not significantly different from zero was observed by almost all investigators suggesting that, in the future, passage from DCC to ER-EIA should not present serious problems for the clinical interpretation of the receptor data. With regard to IEF, the correlation is different (slope 1.9) (Laboratory G). The reason for this observation is unknown and merits further investigation, but lower values found with the IEF compared to the steroid binding method have been reported (26) and thus confirm the observed findings.

The lower ER level found by both methods in premenopausal women compared to postmenopausal women confirms earlier findings and suggests the absence of occupied ER sites in the majority of cytosols from premenopausal women. Depletion of receptors from cytosol by activation after binding to endogenous steroids or modulation of receptors by unknown physiological factors could be responsible for this phenomenon.

For the reasons mentioned above it seems that introduction of the Abbott ER-EIA in routine practice may present a step forward in the characterization of the hormone sensitivity of human mammary tumors. However, this introduction will not solve all technical problems. Factors such as tissue handling, storage, and homogenization, which are also of prominent importance, have to be carefully controlled. It seems therefore that routine receptor determinations should be performed in laboratories with established quality assurance procedures.

References

Abbott Monoclonal Enzyme Immunoassay Measurement of Estrogen Receptors in Human Breast Cancer: A European Multicenter Study


*Cancer Res* 1986;46:4233s-4236s.

Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/8_Supplement/4233s

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.