Quantitation of Estrogen Receptor in Seventy-five Specimens of Breast Cancer: Comparison between an Immunoassay (Abbott ER-EIA Monoclonal) and a \[^3\text{H}\]Estradiol Binding Assay Based on Isoelectric Focusing in Polyacrylamide Gel^1

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Abstract

Quantitation of estrogen receptor has been performed in cytosol prepared from 75 specimens of breast cancer tissue from patients who had not received hormonal therapy. The study was performed in order to compare an immunoassay (Abbott Laboratories, North Chicago, IL) with our currently used method for estrogen receptor analysis based on isoelectric focusing of \[^3\text{H}\]estradiol-receptor complex in polyacrylamide gels. Using linear regression analysis, a regression coefficient (slope) of 1.30 and a correlation coefficient of 0.75 were calculated. The differences in results between the two methods are probably partly explained by the fact that the ligand-based method only measures unoccupied receptor, whereas the immunoassay detects the total amount of receptor, resulting in generally slightly higher concentrations with the latter method. However, in five of 75 specimens the ligand-based method gave a considerably higher concentration of estrogen receptor. This was most probably explained by partial proteolysis resulting in the formation of receptor fragment(s), which was undetectable with the immunoassay but detectable with the ligand-based method. These observations underline the importance of careful handling of specimens during the whole immunoassay procedure.

Introduction

The use of a radioactive steroid as a marker for the receptor protein to which it binds has provided a substantial part of our present knowledge concerning estrogen receptors and their interactions in target cells (1). However, the labeled ligand technique has certain inherent limitations. A need has developed for alternative methods for detection and quantitation of estrogen receptors whether or not they are occupied by endogenous hormone. The recent preparation of specific monoclonal antibodies against the human estrogen receptor (2, 3) and establishment of immunochemical techniques for receptor quantitation provide such an alternative approach for studies of estrogen receptors (4). Moreover, the importance of estrogen receptor measurements in therapeutic decision-making is well established for breast cancer, and the new method may provide a more practical and less time-consuming technique for estrogen receptor determination (5). The present paper describes a comparison between a recently commercially available immunochemical technique and our currently used method for receptor quantitation based on analysis of \[^3\text{H}\]estradiol-receptor complex by isoelectric focusing in polyacrylamide gel (6, 7).

Materials and Methods

Tissue Material. Pieces of mammary carcinomas were cut out immediately after surgery and kept on ice. Within 1 to 2 h the specimens were analyzed or frozen at −70°C. Tissue specimens were thawed quickly, minced, and suspended in approximately 3 volumes of buffer [10 mM Tris-HCl (pH 7.4)-1.5 mM EDTA-1 mM dithiothreitol] at 4°C. The tissue was homogenized using an Ultraturrax, and cytosols were prepared by centrifugation at 105,000 × g for 60 min. Aliquots of the cytosols were frozen at −70°C and later thawed and taken for simultaneous analysis of estrogen receptor with the immunoassay (Abbott Laboratories, North Chicago, IL) and our currently used ligand-based isoelectric focusing method (6, 7), respectively. The 105,000 × g pellet was analyzed for DNA content (8) using calf thymus DNA (Sigma) as a standard. The immunoassay was performed as recommended by the manufacturer, and isoelectric focusing in polyacrylamide gel was performed as described earlier (6, 7). The detection limit for both methods was set to 5 fmol/ml of cytosol, and the obtained values correlated to the concentration of DNA.

After analysis of 75 specimens with both methods it was found that 5 samples revealed higher receptor concentrations with the ligand-based method as compared to the immunoassay (ratio between immunoassay and electrofocusing method, <0.3). Aliquots of cytosol from these samples and 10 randomly chosen samples were thawed and reanalyzed by immunoassay and electrofocusing, respectively. Aliquots of cytosol were analyzed with or without treatment with trypsin. As earlier shown, nontrypsinized estrogen receptor focuses at pl 6.1, while the trypsinized form focuses at pl 6.6 (6). These experiments were performed in order to study if proteolysis had occurred during handling of the specimens.

Results

A comparison between the results obtained with the immunoassay and with the isoelectric focusing procedure is shown in both Table 1 and Fig. 1. Based on analyses performed on 3 successive days the interassay variation for the immunoassay was calculated to be 7%, whereas the corresponding value for the ligand-based technique was 12%. All data were calculated on the mean of duplicate analyses. Statistical analyses using linear regression gave a regression coefficient (slope) of 1.30 and a correlation coefficient of 0.75 (Table 1). The immunoassay technique generally yielded slightly higher values. However, in the case of 5 specimens, the electrofocusing method gave more than 3 times higher values than with the immunoassay. Three of these aberrant specimens were from patients below 55 yr of age. Reanalyses of the five samples displaying higher estrogen receptor concentrations with the electrofocusing method showed that the major peak of the \[^3\text{H}\]estradiol-receptor complex focused at pl 6.6 even without trypsin treatment, indicating that proteolysis had already occurred. This was in contrast to the other samples investigated where treatment with trypsin changed the pl of the \[^3\text{H}\]estradiol receptor peak from 6.1 to 6.6, as earlier shown (6).

Discussion

In general, there was relatively good correlation between receptor values obtained with the immunoassay as compared to...
QUANTITATION OF ESTROGEN RECEPTOR

Fig. 1. Comparison of data obtained by analysis of estrogen receptor (ER) in 75 specimens of mammary carcinoma by immunoassay (Abbott Laboratories) and the ligand-based assay of isoelectric focusing in polyacrylamide gel (IFPAGE).

Table 1 Correlation of estrogen receptor concentrations in mammary carcinomas from patients <55 yr and ≥55 yr detectable by both immunoassay and the ligand-based assay of isoelectric focusing in polyacrylamide gel (n = 53).

<table>
<thead>
<tr>
<th>Estrogen receptor concentrations (fmol/ml cytosol)</th>
<th>&lt;55 yr (n = 17)</th>
<th>≥55 yr (n = 36)</th>
<th>Total (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-EIA IFPAGE</td>
<td>227</td>
<td>172</td>
<td>530</td>
</tr>
<tr>
<td>SD (n - 1)</td>
<td>240</td>
<td>307</td>
<td>796</td>
</tr>
<tr>
<td>SD (n)</td>
<td>233</td>
<td>298</td>
<td>785</td>
</tr>
<tr>
<td>y = a + bx</td>
<td>119</td>
<td>130</td>
<td>113</td>
</tr>
<tr>
<td>a</td>
<td>0.63</td>
<td>1.43</td>
<td>0.75</td>
</tr>
<tr>
<td>b</td>
<td>0.80</td>
<td>0.77</td>
<td>0.75</td>
</tr>
<tr>
<td>Correlation</td>
<td>0.76</td>
<td>0.92</td>
<td>0.88</td>
</tr>
<tr>
<td>Correlation (rank)</td>
<td>0.76</td>
<td>0.92</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Table 1 Correlation of estrogen receptor concentrations in mammary carcinomas from patients <55 yr and ≥55 yr detectable by both immunoassay and the ligand-based assay of isoelectric focusing in polyacrylamide gel (n = 53).

* ER-EIA, estrogen receptor immunoassay; IFPAGE, isoelectric focusing polyacrylamide gel.

the electrofocusing procedure. The slightly higher values usually observed with the immunoassay are probably explained on the basis of the capacity of this method to detect both unoccupied and occupied receptors, whereas the electrofocusing technique depends on the ligand-binding ability of the receptor and hence does not detect already occupied receptors. Only 5 of 75 specimens yielded significantly higher receptor values when analyzed by isoelectric focusing as compared to immunoassay. The pI profile of the [3H]estradiol-receptor complex recorded for these five samples indicated that proteolysis had already occurred leading to the formation of receptor fragments not recognized by the antibodies used in the immunoassay kit (4). The presence of sex hormone binding globulin does not influence quantitation of estradiol-receptor by isoelectric focusing in polyacrylamide gel (7). These results underline the importance of rapid and proper handling of tissue specimens intended for receptor analysis, particularly in the case of immunoassay.

References
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