Enzyme Immunoassay and Scatchard Plot Estimation of Estrogen Receptor in Gynecological Tumors

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Abstract

Tissue samples of three endometrial carcinomas, seven ovarian carcinomas, and 24 mammary carcinomas were analyzed for estrogen receptor (ER) by enzyme immunoassay (EIA) and a conventional dextran-coated charcoal (DCC) method. In addition, ER and progesterone receptor were assayed by DCC only in 68 ovarian carcinoma specimens. All three endometrial carcinoma specimens showed elevated ER values by both assays. As with mammary cancers the ER-EIA values tend to be higher than DCC values. It was intriguing to note that negative Scatchard plot data resulted in residual ER levels in the EIA system. Also four ovarian cancer specimens with negative ER values by the DCC assay had detectable levels by ER-EIA, and three of these four had ER-EIA values less than or equal to 10 fmol/mg of protein. Of the ten breast cancers with negative DCC values, seven were ≤ 10 fmol/mg of protein by the ER-EIA. Good correlation (r = 0.88) between EIA and Scatchard plot data was calculated from ER data of 24 mammary carcinoma tissue samples. Receptor assays in 68 ovarian cancer patients indicate that ER determinations should become a useful tool in the management of patients bearing this carcinoma. In addition, receptor determinations may improve the possibility of predicting which well differentiated Stage I ovarian carcinomas are likely to recur. Present data combine to suggest that ER-EIA may become a useful diagnostic laboratory tool.

Introduction

Estimation of ER is a generally acknowledged parameter for the management of mammary carcinoma patients. In addition, ER was found to be of prognostic value (1) although recent evidence suggests PgR to be more important (2). ER levels were observed in endometrial (3) and ovarian carcinoma (4) previously. The present study was aimed at comparing an EIA method with a conventional Scatchard plot assay system for measurement of ER in specimens from patients with ovarian, endometrial, and mammary carcinoma. In addition, ER and PgR assays were carried out in a group of ovarian carcinoma patients in order to evaluate receptor status in such subjects.

Patients and Methods

Tissue samples of 3 patients with endometrial carcinoma, 7 subjects with ovarian carcinoma, and 24 with mammary carcinoma were analyzed for ER by EIA and a conventional DCC method. Tissue samples from patients with endometrial carcinomas were obtained from unselected subjects. The specimens were from diagnostic curettage tissue samples. Comparison of ER-EIA and Scatchard plot data in ovarian carcinoma specimens was also carried out in unselected patients. Tissue samples were obtained during surgery on patients bearing ovarian carcinomas. In addition, ER and PgR were determined by the conventional DCC method in tissue samples of 68 patients who underwent primary surgery of ovarian carcinoma. These patients were recruited for a multicenter national ovarian carcinoma study project. At the time of presentation 41% of subjects were in Stages I and II, whereas the rest presented with Stages III and IV. At a mean follow-up time of 14 mo, 66% of the patients were still alive.

The ER-EIA was carried out by a solid-phase assay based on a "sandwich" method with materials obtained from Abbott Laboratories, Diagnostic Division, North Chicago, IL. Saturation analysis of ER was performed by a method described previously (3). Briefly, 0.2, 1.0, and 10 nm [³H]estradiol (170 Ci/mmole) was incubated in duplicate with 100 μl of cytosol samples in the presence and absence of a 100-fold excess of diethylstilbestrol at 0–4°C overnight. Total incubation volume was 300 μl. DCC was used as separation method. Computerized evaluation of the number of binding sites and affinity constants was done on saturation data. Interassay coefficient of variation was 20%. The assay system had a limit of sensitivity of 5 fmol/mg of protein. Results of ER and PgR levels < 10 fmol/mg of protein are reported as negative, since no response to endocrine therapy was found in these patients by DCC previously.

Our laboratory participates in the program of the European Quality Assessment of Steroid Receptor Assays (Dr. A. Koenders).

Results

ER data for endometrial carcinoma specimens are summarized in Table 1. Due to the small number of patients no statistical evaluation was possible. It was intriguing to note that negative Scatchard plot data resulted in residual ER levels in the EIA system. This is partly due to the fact that negative Scatchard plot data are reported when the level obtained by the DCC method is below 10 fmol/mg of protein, but other explanations are possible. One explanation for this discrepancy may be a difference in sensitivity. Another explanation could be the fact that the Scatchard plot method determines only free ER. All 3 endometrial carcinoma specimens showed elevated ER values by both assays. As with mammary cancers the ER-EIA values tend to be higher than DCC values. It was interesting to note that all 4 ovarian cancer specimens with negative ER values by the DCC assay had detectable levels by ER-EIA (Table 1). Only 3 of 4 ovarian cancer specimens had ER-EIA values ≤ 10 fmol/mg of protein (Table 1). Of the 10 breast cancers with negative DCC values, 7 were ≤ 10 fmol/mg by the ER-EIA.

Good correlation (r = 0.88) between EIA and Scatchard plot data was calculated from ER data of 24 mammary carcinoma tissue samples (Fig. 1).

Determination of receptor levels in 68 patients with ovarian carcinoma was done by Scatchard plot analysis of DCC data only. These results (Table 2) revealed that 32.4% of tissue samples presented with positive ER and PgR. It was interesting to note that positive ER as well as PgR was recorded in 63% of patients older than 60 yr of age, whereas both receptor reports were positive for 36% of the patients younger than 60 yr (data not shown). A significantly greater percentage of patients who survived had detectable ER and PgR compared to those females who died (Table 3). The mean follow-up time was 14 mo in these patients. In addition, patients who presented at the primary surgery with Stages I and II had a high frequency of positive ER and PgR reports than those subjects who were Stage III and IV (Table 4).

Discussion

Recent evidence (3, 4) suggests that ER determination may be useful not only in the management of patients with mammary...
tumors but also in subjects with endometrial and ovarian carcinoma. A rapid and easy-to-perform assay may be helpful for the routine determination of ER. In addition, the use of clinical chemistry gear instead of nuclear medicine equipment may stimulate the rapid spreading of ER assays. Present results suggest that ER is observable in ovarian and endometrial carcinoma by a simple EIA. In addition, good correlation between EIA and Scatchard plot analysis suggests that EIA of ER may become a valuable aid in therapy planning of hormone-dependent tumors in gynecology, although the notion (2) has to be considered that parallel estimations of ER and PgR are necessary. It has to be considered that in some samples ER values below 10 fmol/mg of protein were assayed by Scatchard plot and were designated as negative in Table 1. In the EIA test these samples also exhibited ER levels of less than 10 fmol/mg of protein, although ER was detectable in all but 2 cases. Therefore, it is necessary to define cut-off levels for the ER-EIA. Furthermore, the correlation coefficient indicates that some discordance will be noted. This is also shown in Fig. 1 in which Scatchard plot and EIA data were correlated. Usually higher ER levels were noted in the EIA system. This may be due to the fact that the enzymatic assay system estimates the total receptor population, whereas the Scatchard plot determines only unoccupied receptors. Thus, clinical studies will be necessary to further evaluate these findings.

Determination of ER seems to be of value in the management of ovarian carcinoma (Tables 2 to 4). The incidence of 32.4% for ER- and PgR-positive patients found in this investigation is lower than that of 48–55% observed for subjects with mammary tumors (1, 5). In addition, older patients were noted to have ER and PgR more frequently than younger subjects with ovarian carcinoma (data not shown). This finding is similar to results obtained in breast cancer patients (5) and may be of benefit for those subjects in whom ER and PgR levels are registered and who cannot be treated with cytotoxic agents. This may be similar to the well-acknowledged strategy of treatment of mammary carcinoma patients. Another point of major interest is the observation that the survival rate of patients with ovarian carcinoma is greater in patients with ER and PgR than of those who lack receptors. The prognostic values of ER and PgR estimations seem to be of equal importance for assessing survival rate and risk of recurrence in breast cancer patients (1, 5). A correlation between receptor levels and tumor stages in ovarian carcinomas was noted. This again stresses the important potential use of receptor determinations in ovarian tumors for selecting patients at high risk. The alternate application of ER and PgR assays could be useful as indicators of physiological differentiation which may well be different from morpho-
logical differentiation. Thus, for example, ER and PgR determinations may improve the possibility of predicting which well-differentiated Stage I ovarian carcinomas are likely to recur. It should be noted that PgR is of importance for a more detailed patient evaluation, since PgR seems to be of greater importance for prognosis of disease-free survival and other parameters (2, 6). Further studies are necessary to fully evaluate ER-EIA.

References

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