Effects of Tamoxifen on Estrogen and Progesterone Receptors in Human Breast Cancer

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ABSTRACT

Twenty patients with primary breast cancer were treated with tamoxifen (10 mg p.o. twice a day) for 1 to 4 weeks. Before and after the tamoxifen administration, tumor specimens were obtained and assayed for estrogen receptors and progesterone receptors (PGR). Total cytosol estrogen receptor (ERC) and occupied nuclear estrogen receptor (ERN) were measured by hydroxylapatite assay, and unoccupied PGR was measured by the dextran-coated charcoal assay. ERC, ERN, and PGR were detectable in 11, 8, and 6 tumors, respectively, before tamoxifen administration. After tamoxifen treatment, ERC decreased in 10 of 11 ERC-positive tumors. Occupied ERN increased in three of five ERN-positive tumors treated with tamoxifen for a short period (1 to 2 weeks), but they decreased in all of three ERN-positive tumors after longer administration (3 to 4 weeks). PGR increased in three of five ERN-positive tumors after short-term tamoxifen treatment, but they decreased in all of three tumors treated by the drug for a longer period. Increased PGR responses were accompanied by an increase of ERN in two of three ERN-positive tumors. These results suggest that tamoxifen interacts with the estrogen receptor system in human breast cancer tissues and may be estrogenic during short treatment, while longer treatment results in an antiestrogenic response.

INTRODUCTION

A correlation between the presence of specific ER’s in breast cancer tissues and the response to endocrine therapy has been studied intensively (9, 14). In Japan, ER’s are detectable in about 60% of human breast cancer tissues, of which about 50% respond to endocrine therapy, whereas only about 10% of ER-negative tumors respond to the therapy (13).

Tamoxifen is a nonsteroidal estrogen antagonist which produces a regression of hormone-dependent mammary tumors in humans and rats, and regression has been observed principally in those tumors containing ER (15, 18, 22, 23). It is thus assumed that the antitumor property of tamoxifen is exerted through the ER system. The precise mechanism of action of tamoxifen is, however, poorly understood.

We examined the changes in the levels of ER and PGR in patients with primary breast cancer before and after tamoxifen administration.

MATERIALS AND METHODS

Patients and Processing of Tissues. Twenty Japanese women with primary carcinoma of the breast were studied. Nine were premenopausal and 11 were postmenopausal. In all patients, a histological diagnosis was available. Specimens were collected both at the time of biopsy and after radical resection of the tumors and were kept at −70°C for up to 2 weeks before the assay. Tamoxifen administration (10 mg p.o. twice a day) was started on the day of biopsy and continued until the day before mastectomy. No other drugs were administered.

Frozen tissues were pulverized and weighed. The following procedures were performed at 0°C, unless otherwise indicated. The powder was homogenized in 2 to 4 volumes of phosphate buffer (5 mM sodium phosphate (pH 7.4)-1 mM monothioglycerol-10% glycerol) with three 10-sec bursts of a Polytron PT-10 homogenizer at the lowest setting. The homogenate was centrifuged at 800 x g for 10 min, and the pellet was washed twice with phosphate buffer. All the supernatants were combined and centrifuged at 105,000 x g for 30 min to obtain cytosol. The washed pellet was resuspended in phosphate buffer and filtered through gauze to remove the unhomogenized mass. The filtrate was centrifuged at 800 x g for 10 min. The nuclear pellet was extracted for 30 min with 4 volumes of 10 mM Tris-HCl (pH 8.5)-1.5 mM EDTA-0.6 mM KC1-1 mM monothioglycerol-10% glycerol buffer. Tubes were vortexed at 10-min intervals during the extraction and then centrifuged at 105,000 x g for 30 min to obtain the supernatant nuclear extract.

Hydroxylapatite ER Assay. This assay was performed by a modification of the method of Garola and McGuire (5). Cytosol (200 μl) diluted with phosphate buffer to a protein concentration of 0.5 to 1.5 mg/ml was added to bovine serum albumin-washed tubes. A hydroxylapatite slurry (Bio-Rad DNA grade; 250 μl; a packed hydroxylapatite:buffer ratio of approximately 0.7) was added to the tubes. The mixture was incubated on ice for 30 min, vortexed every 10 min, and centrifuged at 800 x g for 2 min, and the supernatant was discarded. The pellets were incubated with phosphate buffer and increasing quantities of [3H]estradiol (90 Ci/mm; New England Nuclear, Boston, Mass.; 0.03 to 0.4 pmol). An additional set of tubes with 0.4 pmol [3H]estradiol was incubated with 80 pmol of diethylstilbestrol to determine nonspecific binding. Incubation time and temperature are indicated in chart legends. Following incubation, the pellets were washed twice with phosphate buffer containing 1% Tween 80 and then extracted overnight at room temperature with 2 ml ethanol. The extracts were counted for radioactivity in 5 ml scintillation fluid (4.0 g PPO and 0.5 g POPOP made up to 1 liter with toluene).

PGR Assay. PGR was measured by a modification of the method of Horwitz and McGuire (7). Identical aliquots of cytosol were incubated with different concentrations of [3H]R5020 (85.8 Ci/
mmol; New England Nuclear; 0.015 to 0.2 pmol). An additional set of tubes with 0.2 pmol [3H]R5020 was incubated with 40 pmol of unlabeled R5020 to determine nonspecific binding. After incubation for 15 hr at 0°, unbound hormone was removed by the addition of dextran-coated charcoal suspension (0.25% Norit A-0.0025% dextran in 0.01 M Tris-HCl, pH 8.0) to each tube, which was then further incubated for 30 min, vortexed every 10 min, and centrifuged for 10 min at 1600 X g. An aliquot of the supernatant was counted for radioactivity in modified Bray's scintillation fluid (125 g naphthalene, 7.5 g POP, and 0.377 g POPOP made up to 1 liter with dioxane). For both steroids, binding data were calculated and analyzed according to the method of Scatchard (21) with a computer-assisted system. Protein concentration was initially determined by UV absorption (11) and later confirmed by the method of Lowry et al. (12). DNA was determined according to the method of Burton (1).

RESULTS

Ligand Exchange on Hydroxylapatite-adsorbed ER. After the nuclear extract was charged with unlabeled estradiol at 0°, [3H]estradiol binding to hydroxylapatite-adsorbed ERN was minimal when incubated at 0°. When the incubation temperature was raised to 30°, a remarkable increase in the binding of [3H]estradiol to ERN was evident. When excess unlabeled estradiol was added after 2 hr incubation at 30°, [3H]estradiol binding rapidly decreased, suggesting that [3H]estradiol bound at 30° remained exchangeable (Chart 1).

Effects of Time and Temperature on Binding of [3H]Estradiol to Hydroxylapatite-adsorbed ER's of Human Breast Cancer Tissue. Chart 2 illustrates the effect of time and temperature on binding of [3H]estradiol to ER in nuclear extract of human breast cancer tissue. Specific binding of [3H]estradiol to hydroxylapatite-adsorbed ERN at 0° was almost complete within 8 hr and remained nearly constant up to 18 hr. On the other hand, [3H]estradiol binding at 30° was complete within 2 to 3 hr and then gradually decreased. The 30° assay is generally considered to be capable of measuring total receptors. It can be assumed, therefore, that the difference between the 30° and 0° assays represents occupied receptors. A representative Scatchard plot is shown in Chart 3.

Effects of Tamoxifen on ER and PGR of Human Breast Tumors. When the maximum binding site of receptors was less than 30 fmol/mg DNA, the results were interpreted as undetectable. Total ERC was detectable in 11 of 20 tumors and occupied ERC was detectable in 8 tumors before tamoxifen administration (Table 1). All ERN-positive tumors contained ERC. After tamoxifen administration, the amount of total ERC decreased in 10 of the 11 ERC-positive tumors and was undetectable in 5 of them. The amount of ERC fell to less than one-tenth of control values in 4 of 8 ERC-positive tumors after a short period (1 to 2 weeks) of tamoxifen treatment, whereas it fell in all of 3 tumors after longer drug administration (3 to 4 weeks) (Chart 4). The amount of occupied ERC increased in 3 of the 5 ERC-positive tumors after short-term tamoxifen treatment. After a longer period of treatment, however, occupied ERC decreased in all of the 3 ERC-positive tumors (Chart 5). All ER-negative tumors remained ER negative even after tamoxifen administration.

PGR in cytosol were detectable in 6 of 20 tumors before tamoxifen administration. The amount of PGR increased (more than 200 fmol/mg DNA) in 3 of the 5 ERC-positive tumors after
Table 1
Total ERC, occupied ERN, and unoccupied PGR in 11 ERC-positive human breast cancer tissues before and after tamoxifen (20 mg/day)

Tamoxifen was given with no other therapy in patients with primary breast cancer for 1 to 4 weeks. Specimens collected at the time of biopsy and radical resection of the tumors are shown as before and after tamoxifen, respectively. Occupied receptor sites were determined by the difference between total sites measured at 30° and unoccupied sites measured at 0°.

<table>
<thead>
<tr>
<th>Case</th>
<th>Patient age (yr)</th>
<th>Duration of tamoxifen treatment (wk)</th>
<th>Hormone receptors (fmol/mg DNA)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Before tamoxifen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ERC</td>
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<tr>
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<td>1</td>
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<tr>
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<tr>
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<td>57</td>
<td>1</td>
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</tr>
<tr>
<td>4b</td>
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<td>1</td>
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<tr>
<td>11b</td>
<td>52</td>
<td>4</td>
<td>1480</td>
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</table>

* a: 0 < 30 fmol/mg DNA.
* b: Premenopausal patients.

Chart 4. Total ERC in 11 ERC-positive human breast tumors before and after tamoxifen administration (20 mg/day).

Chart 5. Occupied ERN in 8 ERN-positive human breast tumors before and after tamoxifen administration (20 mg/day).

Chart 6. Cytosol unoccupied PGR in 7 PGR-positive human breast tumors before and after tamoxifen administration (20 mg/day).

short-term (1 to 2 weeks) of tamoxifen treatment. In one tumor (Case 5), PGR changed from undetectable to positive after tamoxifen treatment (Table 1; Chart 6). Increased PGR responses were accompanied by an increase of occupied ERN in the ERN-positive tumors (Table 1, Cases 1 and 5). After longer administration of tamoxifen (3 to 4 weeks), however, the amount of PGR decreased in all of the 3 ERN-positive tumors. ERN were also decreased in these tumors after tamoxifen administration (Chart 5).

Dissociation constant of ER and PGR did not change significantly after the tamoxifen administration.

DISCUSSION

It has been reported that the antiestrogenic agent tamoxifen was effective in late or recurrent human breast carcinoma (2, 20, 22, 23). Like other antiestrogens, tamoxifen is much more effective in ER-positive tumors (15). Several mechanisms are considered to be involved in the mode of antitumor action of...
tamoxifen: (a) tamoxifen inhibits the formation of the estrogen-receptor complex; (b) the drug substitutes a receptor complex of lower intrinsic biological activity than that of the estrogen-receptor complex; (c) with continued administration of tamoxifen, the concentration of ERC decreases (17). It is generally accepted that tamoxifen binds to ERC and that the antiestrogen-receptor complex translocates into the nucleus in mammary tumors. However, the precise sequence of events has not been fully elucidated.

In our study, we measured ER by the hydroxylapatite assay of Garola and McGuire (5). They showed the presence of nuclear protease in nuclear extracts of human breast tumors (4). They also demonstrated that the effect of nuclear proteolytic activity could be eliminated by adsorbing the receptors to hydroxylapatite, prior to the addition of radioactive ligand and warming. They showed that at 30° the binding reached a maximum after 3 hr and was stable for 7 hr (5). In our experiments, [3H]estradiol binding to hydroxylapatite-adsorbed ERN at 30° reached a maximum after 2 to 3 hr and then gradually decreased (Chart 2), suggesting that the protection from the nuclear proteolytic activities seems to be incomplete in our system. Geier et al. (6) showed that receptor degeneration by nuclear proteolytic activity at 30° could be avoided by repeated washing of the particulate-bound receptor.

Using the exchange assay, both occupied and unoccupied ERN’s were detectable in nuclear extracts (5, 25). It was reported that unoccupied ERN’s were positive in about one-half of the ERC-positive breast tumors (5, 6, 19). We obtained similar results (24). However, Edwards et al. (3) have recently shown that the unoccupied ERN value may represent the cytoplasmic contamination of the crude nuclear pellet used in the assay. In contrast, the amount of occupied ERN found in nuclear preparations was not considerably affected by contamination of the preparation with cytoplasmic receptors (3). Considering these technical problems to be solved, we described only occupied ERN in the present study.

It was reported in human breast cancer cells (MCF-7) that, with increasing doses of tamoxifen, ERC’s were progressively depleted and translocated into the nucleus (10). In the present study, the amount of ERC decreased after the tamoxifen administration in human breast cancer. Occupied ERN levels were rather increased after short-term tamoxifen treatment but decreased after longer administration of the drug.

Horwitz et al. (8, 10) have shown in MCF-7 cells that tamoxifen has dual dose-dependent estrogenic-antiestrogenic properties. At lower doses, tamoxifen is a potent estrogen and rapidly induces PGR. However, at higher levels, the drug is an antiestrogen, and cell growth and PGR induction are suppressed. Tamoxifen treatment of metastatic breast cancer may be associated with transient exacerbation of bone pain or skin lesions. Plotkin et al. (20) designated this phenomenon as the tamoxifen flare, which usually began within 2 to 10 days after the start of tamoxifen therapy and subsided during a period for 1 to 4 weeks while drug treatment continued. Recently, Nam et al. (16) reported that, after 1 week of treatment with tamoxifen (30 mg/day), PGR increased in 6 of 14 postmenopausal patients with metastatic breast cancer containing ERC. In the present study, we observed that the amount of PGR increased after short-term (1 to 2 weeks) tamoxifen (20 mg/day) whereas, after longer administration (3 to 4 weeks) of the drug, PGR decreased in primary breast cancer.

It is suggested, therefore, that tamoxifen interacts with the ER system in human breast cancer tissues and may be estrogenic during short-term administration (1 to 2 weeks) while it may require a longer period of time for the in vivo concentration of the drug to build up to antioestrogen levels. In this study, breast tumors were resected completely after 1 to 4 weeks of tamoxifen administration if tumor regression was not observed. The relationship between the changes of hormone receptors and antitumor effect in human breast cancer after a longer period of tamoxifen administration is now under investigation.

ACKNOWLEDGMENTS

We are grateful to Drs. K. Matsumoto, H. Sugano, and O. Abe for pertinent advice; to Dr. W. L. McGuire for instruction in techniques; to I.C.I. Co. for providing tamoxifen; and to M. Ohara for reading the manuscript.

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