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Influence of Tamoxifen-Medroxyprogesterone Sequential Therapy on Estrogen and Progesterone Receptor Contents of Breast Cancer

Shinzaburo Noguchi, Hitoshi Yamamoto, Hideo Inaji, Shingi Imaoka and Hiroki Koyama

Department of Surgery, The Center for Adult Diseases, Osaka, 3-3 Nakamichi 1-chome, Higashinari-ku, Osaka 537

The influence of tamoxifen (TAM) and medroxyprogesterone acetate (MPA) sequential administration on the estrogen receptor (ER) and progesterone receptor (PR) contents of breast cancer was studied in 68 patients with operable breast cancer. TAM was used as a primer of PR induction in order to enhance the effects of MPA. Half of the patients (n=34) were preoperatively treated with TAM (20 mg/day for 7 days) and sequentially with MPA (1200 mg/day for 17 (median) days). ER and PR of surgical specimens were assayed by enzyme immunoassay and the results were compared with those obtained from the other half of the patients (n=34), who had not received any treatment before surgery. TAM-MPA treatment significantly lowered PR in the cytosol regardless of the menopausal status. On the other hand, TAM-MPA treatment significantly lowered ER in the cytosol only in the postmenopausals but not in the premenopausals. These results demonstrate that reduction of ER provoked by TAM-MPA treatment is dependent on menopausal status.

Key words: Tamoxifen — Medroxyprogesterone — Estrogen receptor — Progesterone receptor

Recently, tamoxifen(TAM)-medroxyprogesterone acetate (MPA) sequential therapy has been introduced as a new combination endocrine therapy for breast cancer. The rationale that this therapy is based on is to sensitize breast cancer cells to MPA by inducing progesterone receptors with TAM pretreatment.[1] Iacobelli *et al.*[2] have demonstrated that the growth-inhibitory effect of MPA on breast cancer cells (CG-5) can be augmented by priming these cells with TAM. Tominaga *et al.*[3] have confirmed the superiority of TAM-MPA sequential therapy over TAM or MPA alone in 7,12-dimethylbenz-[*a*]anthracene-induced rat mammary tumors.

The clinical usefulness of TAM-MPA therapy for breast cancer is currently under evaluation. A few preliminary studies have indicated that TAM-MPA therapy confers a better response rate than TAM therapy. Garcia-Giralt *et al.*[4] reported that the response rate to TAM-MPA therapy (60%) was higher than that to TAM therapy (48%). Gundersen *et al.*[5] also reported an improved response to TAM-MPA compared to TAM (69% vs. 27%). This new combination endocrine therapy seems attractive, and there are several ongoing trials using TAM-MPA therapy in modified forms.

However, several important questions still remain unanswered in order to carry out TAM-MPA therapy most effectively. One of them is whether or not ER can be replenished after one cycle of TAM-MPA administration. While it is well established that MPA decreases ER of human endometrium,[6] this effect has rarely been studied on human mammary cells. The priming effect of TAM can be expected only when tumors contain ER. Thus, if the tumors lose ER after one cycle of TAM-MPA administration, subsequently administered TAM cannot exert the PR-inducing property. Therefore, it seems quite important to investigate the behavior of ER over time during TAM-MPA therapy.

In this study, changes of ER and PR contents of breast cancers were studied after one cycle of TAM-MPA treatment. We have found that one cycle of TAM-MPA treatment decreases PR irrespective of menopausal status and decreases ER in postmenopausals but not in premenopausals.

MATERIALS AND METHODS

Patients and treatment schedules Sixty-eight patients with operable breast cancer entered this study. Half of them (n=34) were preoperatively treated with 20 mg of TAM for 7 days and sequentially with 1200 mg of MPA for 17 (median) days, ranging from 11 to 28 days, until the day before surgery (TAM-MPA group). The other half of the patients (n=34) did not receive any treatment before surgery (control group). The TAM-MPA and control groups were both composed of 16 premenopausals and 18 postmenopausals. Before TAM-MPA administration, serum estradiol and gonadotropin (LH and FSH) levels were examined in every patient. Patients who were actually menstruating with elevated estradiol levels were considered as premenopausals and those who were not menstruating with low estradiol and high gonadotropin levels were considered as postmenopausals. Perimenopausal patients were excluded from this study because of the unstable hormonal milieu. In the TAM-MPA group, premenopausals received MPA

treatment for 18 (median) days, ranging from 11 to 28 days, and postmenopausals received MPA treatment for 16 (median) days, ranging from 12 to 26 days.

Tumor specimens removed at surgery were kept at −80°C until assay. In all patients a histologic diagnosis of infiltrating ductal carcinoma was obtained.

Preparation of cytosols and nuclear extracts Every procedure was carried out at 0–4°C unless otherwise specified. Surgical specimens were homogenized in 5 volumes of TEDMG buffer (10 m*M* Tris, 1.5 m*M* EDTA, 0.5 m*M* dithiothreitol, 10 m*M* sodium molybdate, 10% (v/v) glycerol, pH 7.4) using a Polytron P-10 (Brinkmann Instruments, Westbury, NY) set at 4, with 10 s runs and a 30 s cooling period between each run. An aliquot (200 μl) of the homogenate was taken for DNA assay, and centrifuged at 800*g* for 10 min. The supernatant was removed and the pellet was resuspended in 5 volumes of TEDMG buffer. After centrifugation at 800*g* for 10 min, the supernatant was removed, combined with the former supernatant, and centrifuged at 105,000*g* for 60 min. The resultant supernatant was obtained as cytosol without a superficial lipid layer.

The washed pellet was extracted in 5 volmes of TEDMGK (TEDMG plus 0.6 *M* KCl) for 60 min. The crude nuclear extract was centrifuged at 105,000*g* for 60 min and the resultant supernatant was obtained as nuclear extract.

Enzyme immunoassay for ER and PR Enzyme immunoassay (EIA) kits for ER were purchased from, and those for PR were generous gifts from, Dainabot Laboratories (Tokyo). All the procedures for ER-EIA and PR-EIA were carried out according to the method previously described by us.[7] Intra- and inter-assay coefficients of variation (%CV) of ER-EIA were 5.6% and 4.1%, respectively, and those of PR-EIA were 8.7% and 7.9%, respectively. Every sample of cytosol and nuclear extract was diluted two-fold with TEDMG buffer and assayed for ER and PR in duplicate.

Protein and DNA were assayed according to the methods of Lowry *et al.*[8] and Burton,[9] respectively.

Statistical analysis The significance of differences of the mean receptor values was examined by means of the *t* test after logarithmic conversion of each value.

RESULTS

Influence of TAM-MPA administration on PR contents The influence of TAM-MPA administration on PR contents of the tumors was studied qualitatively with a cut-off value of 100 fmol/mg DNA. A tumor was considered PR-positive when the total of PR in the cytosol (PRc) and nuclear (PRn) fractions was above 100 fmol/mg DNA.[10] PR positivity tended to decrease after TAM-MPA administration. PR positivity was 56% (9/16) for premenopausals and 50% (9/18) for postmenopausals in the control group but it decreased to 38% (6/16) for premenopausals and 22% (4/18) for postmenopausals after one cycle of TAM-MPA administration (TAM-MPA group).

The influence of TAM-MPA administration on PR contents was also studied quantitatively. The mean PRc and PRn values of PR-positive tumors are shown according to menopausal status in Fig. 1. No significant difference was found in the PRn value between the control and TAM-MPA groups, irrespective of menopausal status. On the other hand, the PRc value in the TAM-MPA group was significantly lower than that in the control group and the decrease in the PRc value was more pronounced in the postmenopausals as compared to premenopausals.

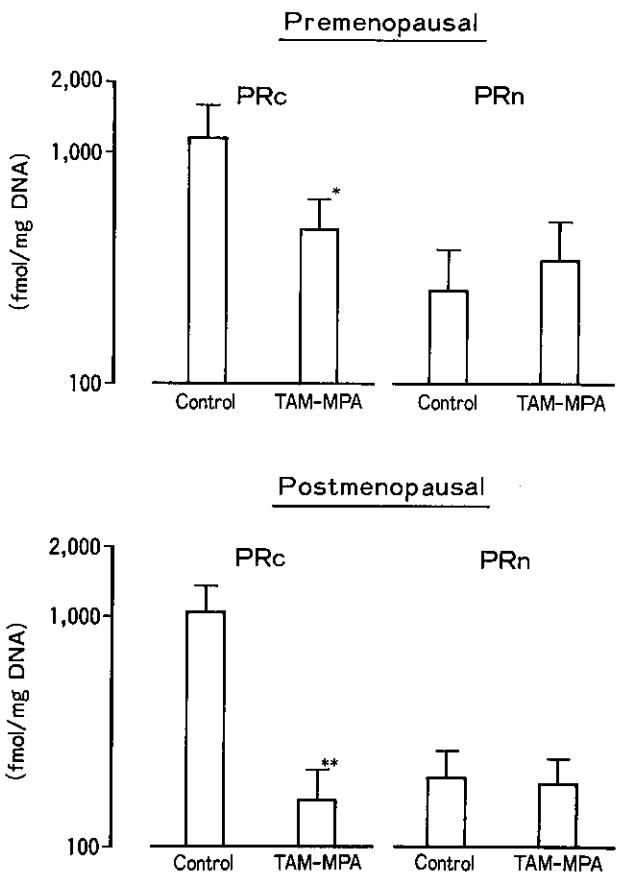

Fig. 1. Influence of TAM-MPA administration on PR contents of the tumors. PRc (cytosol fraction) and PRn (nuclear fraction) were assayed by EIA as described in "Materials and Methods." * $P<0.05$ and ** $P<0.01$ when compared to the values of the control group. Each value is the mean of PR-positive tumors; bars, SE.

Influence of TAM-MPA administration on ER contents The influence of TAM-MPA administration on the ER contents of the tumors was studied qualitatively. A tumor was considered ER-positive when the total of ER in the cytosol (ERc) and nuclear (ERn) fractions was above 100 fmol/mg DNA.[10] ER positivity was 63% (10/16) for premenopausals and 67% (12/18) for postmenopausals in the control group and 69% (11/16) for premenopausals and 50% (9/18) for postmenopausals in the TAM-MPA group. No significant difference was found in ER positivity between the control and TAM-MPA groups.

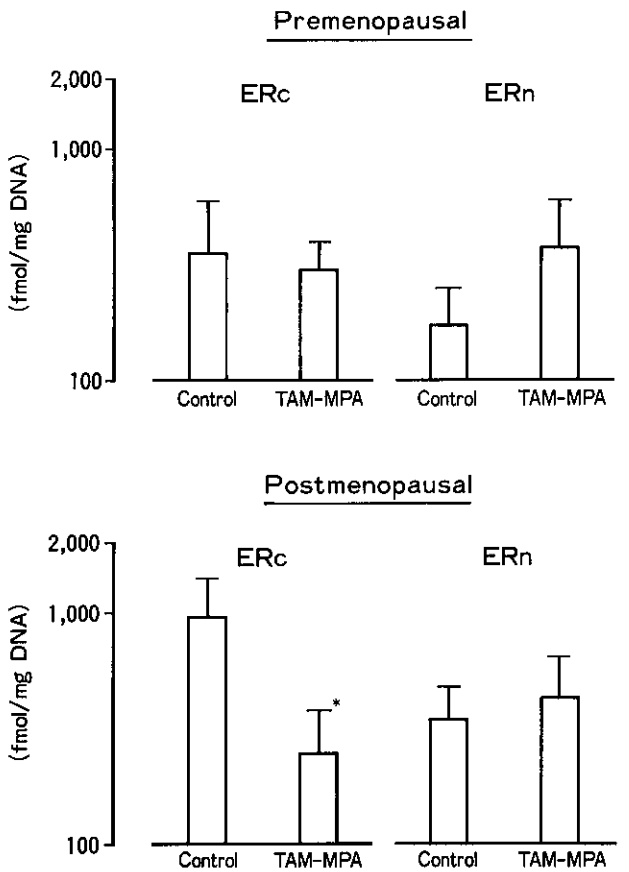

Fig. 2. Influence of TAM-MPA administration on ER contents of the tumors. ERc (cytosol fraction) and ERn (nuclear fraction) were assayed by EIA as described in "Materials and Methods." * $P < 0.05$ when compared to the values of the control group. Each value is the mean of ER-positive tumors; bars, SE.

The influence of TAM-MPA administration on ER contents was also studied quantitatively. The mean ERc and PRc values of ER-positive tumors are shown according to menopausal status in Fig. 2. In premenopausals, no significant difference was found in ERc and ERn between the control and TAM-MPA groups. However, ERc decreased significantly after TAM-MPA administration in postmenopausals while ERn was not affected.

Influence of TAM-MPA administration on ER and PR distribution in cytosol and nuclear fractions The distribution of ER and PR in the cytosol and nuclear fractions were compared between the control and TAM-MPA groups. Percentages of ERn and PRn were calculated from ER- and PR-positive tumors, respectively (Table I). Both ERn and PRn tended to increase after TAM-MPA administration, irrespective of menopausal status, while this effect was more pronounced in postmenopausals.

DISCUSSION

Because ER and PR in the cytosol and nuclear fractions were assayed immediately after TAM-MPA administration, most receptors are considered to be in the occupied form. Therefore, we assayed these receptors with an EIA which can detect the receptors whether or not they are occupied with the corresponding hormones.[7,11] Conventional exchange assay has been a method of choice for the detection of receptors in the occupied form but this method is onerous and sensitive to procedural details. On the other hand, recently developed EIA for ER and PR appears to be best suited for the detection of receptors in the occupied form because of its methodological simplicity and excellent reproducibility.

Table I. Comparison of ER and PR Distribution in Cytosol and Nuclear Fractions between Control and TAM-MPA Groups

	Control		TAM-MPA	
	ER	PR	ER	PR
Premenopausal	41.4±7.8[a]	23.1±7.3	56.5±7.5	38.2±4.6
Postmenopausal	28.6±5.2	16.9±1.2	59.6±8.6[b]	35.6±4.7[b]

a) Mean percentages of nuclear receptors±SE.
b) $P < 0.05$ when compared to the control values.

Recently it has been shown that both ER and PR are exclusively located in the nucleus.[12, 13] Thus, the receptors detected in the cytosol are considered to be migrants from the nucleus during homogenization. However, separate determination of the cytosolic and nuclear receptors is still meaningful for the following reasons. Unoccupied receptors are thought to migrate more easily from the nucleus than are occupied receptors, since unoccupied receptors bind loosely and occupied receptors bind tightly to DNA. Therefore, cytosolic and nuclear receptors appear to represent unoccupied and occupied receptors, respectively. It seems useful to determine the cytosolic and nuclear receptors separately in order to estimate the degree of receptor occupancy.

We have already demonstrated that priming for one week with TAM significantly increases PR in breast cancers.[10] Therefore, the effect of MPA which was given after priming with TAM should have been augmented. This situation seems convenient for the study of MPA action since the effect of MPA is expected to appear in an enhanced form.

The duration of MPA treatment employed in this study was relatively short, because we wished to examine the short-term effect of MPA on ER and PR contents. Long MPA treatment is known to induce tumor regression in 30–40% of patients. In regressing tumors, degeneration and necrosis of tumor cells must take place and affect the receptor levels non-specifically. This situation makes it more difficult to elucidate the action mechanism through which MPA exerts its antiproliferative effects. In this study, neither tumor regression nor degenerative change in tumor tissue was observed in any patient after one cycle of TAM-MPA treatment. This fact seems to support the thesis that MPA treatment for 17 (11–28) days is appropriate for the examination of the short-term effect of MPA.

TAM-MPA decreased PRc without a concomitant increase in PRn, resulting in a decrease in total PR (Fig. 1). This result cannot be explained only by the nuclear translocation of PR. Nuclear processing of PR, described by Horwitz *et al.*,[14] seems responsible for this decrease in total PR. Though TAM-MPA decreased PRc irrespective of menopausal status, this effects was more pronounced in postmenopausals. Induction of PR with endogenous estrogens in premenopausals may account for this difference.

TAM-MPA did not affect the ER contents so markedly. A slight but significant decrease in ERc was found only in postmenopausals but not in premenopausals after TAM-MPA administration (Fig. 2). This result is consistent with that reported by Lundgren *et al.*[15] They measured ER of recurrent tumors of four postmenopausal patients sequentially before and after MPA treatment and found that ERc decreased by only 18% one week after the start of MPA treatment. These results demonstrate that short-term treatment with MPA decreases ERc of breast cencer only to a slight extent and that reduction of ERc is probably not a major pathway through which MPA exerts its antiproliferative effect. MPA seems to inhibit the breast cancer cell growth mainly through different mechanisms, such as suppression of the adrenocortical axis and direct toxic actions.

The finding that TAM-MPA decreased ERc in postmenopausals but not in premenopausals is partially attributable to the role played by endogenous estrogens and progesterone in premenopausals. Estrogens are supposed to intervene in the action of TAM and MPA, and furthermore have a direct effect to lower ERc by translocating ERc into the nucleus. Progesterone also seems to play an important role in lowering ERc for the following reasons. Smyth *et al.*[16] and Saez *et al.*[17] reported that the ER contents fell during the early secretory phase, when the serum progesterone level was rising, and hypothesized that the cyclic increase in progesterone limits the ER synthesis. The well documented phenomenon that the ER positivity of breast cancer is higher in postmenopausals than in premenopausals may also be explained by a role played by endogenous progesterone in premenopausals. Therefore, we can assume that the ER level in premenopausals is originally kept low by endogenous estrogens and progesterone and that MPA is unlikely to elicit additional inhibitory effects on the ER synthesis. However, MPA more easily affects the ER synthesis in postmenopausals because breast cancers are not exposed to endogenous progesterone. Interestingly, the ER value of postmenopausals in the TAM-MPA group was quite similar to that of premenopausals in the control group. These results might represent additional support for the view that endogenous progesterone suppresses the ER synthesis in premenopausals.

The increase in percentage of ERn after TAM-MPA administration was an unexpected observation (Table I). Since MPA lacks affinity to ER, nuclear translocation of ERc is unlikely to be induced by MPA even at high doses. A possible explanation is that TAM, which was given before MPA, remains to affect the ER distribution and inhibits ER replenishment due to its long pharmacokinetic half life (4–7 days).[18]

It has been argued that a washout period between each cycle is necessary for the replenishment of ERc in order to carry out TAM-MPA therapy more effectively. Our results demonstrate that breast cancers contain enough ERc for subsequent priming with TAM after one cycle of TAM-MPA therapy. Therefore, we think a washout period is unnecessary in order to produce ER replenishment.

In conclusion, our results demonstrate that short-term treatment with MPA after TAM-priming reduces ERc in

postmenopausals but not in premenopausals, probably due to the interference of endogenous estrogens and progesterone. However, we can not rule out the possibility that long-term treatment with MPA might reduce ERc even in premenopausals. Our study is vulnerable to criticism on the issue that not only MPA-responsive but also MPA-nonresponsive tumors were included in the analysis of ER levels, i.e., changes in ER levels were studied on ER-positive tumors, but it is well known that about a half of ER-positive tumor do not respond to MPA. Therefore, the effect of MPA on ER levels must have been underestimated due to the inclusion of non-responsive tumors. Ideally, the effect of MPA should be studied only on MPA-responsive tumors. At present, however, it is impossible to distinguish MPA-responsive from MPA-nonresponsive tumors with accuracy before or even after short-term treatment since short-term treatment is not enough for the assessment of tumor regression. If an accurate marker for predicting the responsiveness to MPA treatment were available, these problems would be solved and a more conclusive study on the action mechanism of MPA could be made.

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