Inability of Medroxyprogesterone Acetate to Down Regulate Estrogen Receptor Level in Human Breast Cancer

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

The influence of medroxyprogesterone acetate (MPA) on estrogen receptor (ER) and progesterone receptor (PR) levels was studied in 20 postmenopausal patients with ER-positive and PR-positive primary breast cancers. Each patient underwent drill biopsy and subsequently mastectomy. The drill biopsy and surgical specimens were assayed for the total ER and PR levels (cytosolic plus nuclear fraction) by enzyme immunoassay. Between the drill biopsy and mastectomy, ten patients received no treatment (control group) and the other ten patients were given MPA (1200 mg/day) for 7 days. In the control group, the total ER and PR levels of the surgical specimens decreased by 68.2 ± 7.3% and 60.7 ± 8.4%, respectively, taking the receptor values of the drill biopsy specimens as 100%, although no treatment was given preoperatively. This decrease seems to be attributable to the receptor degradation due to damages occurring during mastectomy. In the MPA group, the total ER and PR levels of the surgical specimens decreased by 64.2 ± 8.0% and 23.3 ± 7.6%, respectively. The decrease in PR, but not ER, was statistically significant between the control and MPA groups ($P < 0.01$). These results demonstrate that MPA down regulates PR but not ER in human breast cancer and challenge the conventional idea, extrapolated from the results on the endometrium and endometrial cancer, that MPA antagonizes endogenous estrogens by down regulating ER. *Cancer* 65:1375–1379, 1990.

RECENTLY, medroxyprogesterone acetate (MPA) has proven to be a useful endocrine treatment for breast cancer. The response rate achieved with high-dose MPA treatment is reported to be 30% to 40%.[1] Medroxyprogesterone acetate appears advantageous over tamoxifen (TAM) in terms of antitumor effects since MPA can be still of benefit to patients who fail to respond to or become resistant to TAM, but not *vice versa*.[1] This fact demonstrates that MPA exerts its effects through pathways different from those involved in TAM action.

The superiority of antitumor effects of MPA over TAM is postulated to be explained by a dual mechanism of MPA action: (1) direct effect through progesterone receptor (PR); and (2) indirect effect through suppression of gonadotropin and adrenocorticotropic hormone (ACTH) secretion from the pituitary gland, resulting in estrogen deprivation. The indirect effect of MPA has been well established. Medroxyprogesterone acetate decreases the secretion of gonadotropin and ACTH within a few weeks.[2,3] Wander *et al.* reported that antitumor activity of MPA coincided with the extent of endogenous cortisol suppression.[4] This fact strongly suggests that the suppression of ACTH secretion plays an important role in the antitumor effects of MPA in postmenopausal patients. On the other hand, the direct effect of MPA on breast cancer through PR is still unclear although it is well established in the endometrium and endometrial cancer. Medroxyprogesterone acetate down regulates estrogen receptor (ER)[5,6] and induces 17β-estradiol dehydrogenase (E_2DH) which converts estradiol to biologically less active estrone, resulting in refractoriness to endogenous estrogens.[6,7] These mechanisms are considered to play an important role in promoting growth inhibition of endometrial cancer. Teulings *et al.*, however, reported that pro-

From the Department of Surgery, The Center for Adult Diseases, Osaka, Japan.

Address for reprints: Shinzaburo Noguchi, MD, Department of Surgery, The Center for Adult Diseases, Osaka, 3-Nakamichi 1-Chome, Higashinari-ku, Osaka 537, Japan.

Accepted for publication October 17, 1989.

gestins (megestrol acetate) did not increase the activity of E_2DH in human breast cancer.[8] This fact suggests that the action mechanism of MPA on breast cancer might be different from those postulated on endometrial cancer.

Since MPA has been demonstrated to down regulate ER in the endometrium[5] and endometrial cancer,[6] it is of interest to study whether or not MPA can down regulate ER in breast cancer for the purpose of elucidating the tissue-related difference of MPA action. Unfortunately, this problem has rarely been studied and only preliminary results are available.[9] In this study, we investigate the influence of MPA treatment on ER levels and its nuclear and cytosolic distribution and have found that, unlike in the endometrium and endometrial cancer, MPA neither down regulates ER nor changes its distribution in breast cancer.

Materials and Methods

Treatment Schedule

Postmenopausal patients with a breast tumor measuring 2 to 3 cm in diameter and with positive results of mammography, ultrasonography, and fine-needle aspiration cytologic study of the tumor were consecutively entered in this study from September 1988 to April 1989. In all, 48 patients were enrolled in this study and an informed consent was obtained from every patient. Ten to 12 days before surgery, those patients received drill biopsy of the breast tumor. The drill biopsy specimens were assayed for ER and PR by enzyme immunoassay described below and only patients with both ER-positive and PR-positive tumors (> 100 fmol/mg DNA) were further treated following the schedule mentioned below. Patients with ER-negative and/or PR-negative tumors were excluded from this study. Patient entry ceased when the number of ER-positive and PR-positive tumors reached 20. The first ten patients received no treatment before surgery (control group) and the second ten patients were orally given 400 mg MPA three times daily for 7 days until the day before surgery (MPA group).

Drill biopsy of breast tumor was performed according to the method we previously described.[10] The mean wet weight of drill biopsy specimens was 35 ± 5 mg (SE). After mastectomy, surgical specimens were obtained from the removed breasts. A histologic diagnosis of infiltrating ductal carcinoma was obtained from each of the 20 patients.

Assay Techniques

Enzyme immunoassay for estrogen receptor and progesterone receptor in cytosol and nuclear extracts: Preparations of cytosolic and nuclear extracts were carried out according to the method previously described.[10] In brief, specimens were homogenized by teflon glass homogenizer in TEDMG buffer (10 mmol/l Tris, 1.5 mmol/l ethylenediamine tetraacetic acid [EDTA], 0.5 mmol/l dithiothreitol, 10 mmol/l sodium molybdate, 10% [v/v] glycerin, pH 7.4), 0.5 ml for the drill biopsy specimen and 5-volume for the surgical specimen. The homogenate was centrifuged at 800 × *g* for 10 minutes. Aspiration was done of the supernatant, and the pellet was washed with TEDMG buffer, 0.5 ml for the drill biopsy specimen and 5-volume for the surgical specimen, and centrifuged at 800 × *g* for 10 minutes. The second supernatant was combined with the first supernatant and centrifuged at 105,000 × *g* for 60 minutes. The resultant supernatant without lipid layer was obtained as cytosol.

The 800 × *g* pellet was extracted with TEDMGK buffer (TEDMG plus 0.6 mol/l potassium chloride [KCl], pH 7.4), 1 ml for the drill biopsy specimen and 5-volume for the surgical specimen, for 60 minutes at 4°C, with a vortex at 10-minute intervals. After extraction, the mixture was centrifuged at 105,000 × *g* for 60 minutes and the resultant supernatant was used as the nuclear extract.

Enzyme immunoassay (EIA) for ER and PR of the cytosol and nuclear extracts were performed according to the methods previously described,[10,11] using ER-EIA and PR-EIA kits purchased from Abbott Laboratory (North Chicago, IL).

Other assays: DNA and protein were assayed according to the methods of Burton[12] and Lowry.[13]

Results

Influence of Medroxyprogesterone Acetate Treatment on Estrogen Receptor and Progesterone Receptor Levels

Changes in total PR levels (cytosol plus nuclear fractions) between the drill biopsy and surgical specimens are illustrated on an individual basis in Figure 1. A significant difference of PR levels between the drill biopsy and surgical specimens was found both in the control and MPA groups ($P < 0.01$, rank sum test). The degree of decrease is compared between the control and MPA groups in Table 1, where the PR value of the surgical specimen is expressed as percentages, taking the PR value of the drill biopsy specimen as 100%. The percentage of PR level (23.3 ± 7.6%) in the MPA group was significantly lower than that (60.7 ± 8.4%) in the control group ($P < 0.01$, *t* test).

Changes in total ER levels (cytosol plus nuclear fractions) between the control and MPA groups are illustrated on an individual basis in Figure 2. A significant difference between the drill biopsy and surgical specimens was found both in the control and MPA groups ($P < 0.01$, rank sum test). The degree of decrease is compared between the control and MPA groups in Table 1, where the ER value of the surgical specimen is expressed as percentages, taking

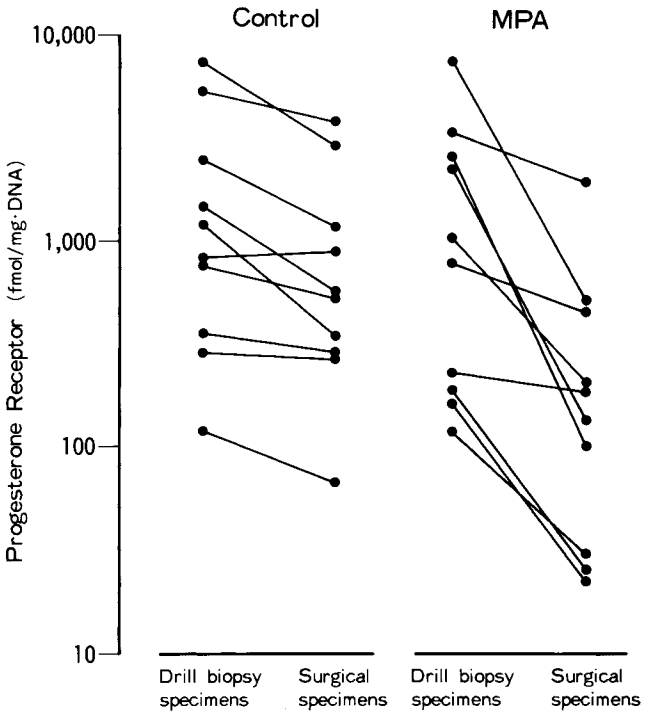

FIG. 1. Changes in total PR levels (fmol/mg DNA) between drill biopsy and surgical specimens on an individual basis in the control and MPA groups.

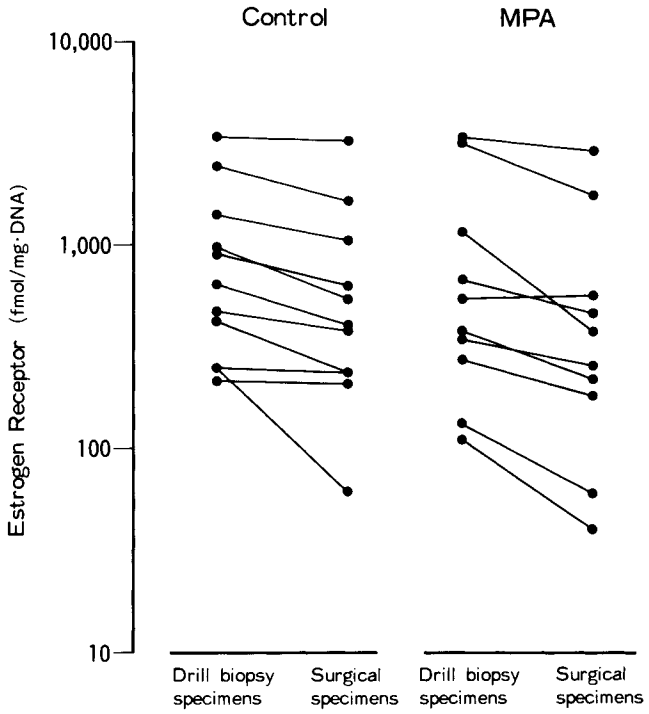

FIG. 2. Changes in total ER levels (fmol/mg DNA) between drill biopsy and surgical specimens on an individual basis in the control and MPA groups.

the ER value of the drill biopsy specimen as 100%. Unlike the result obtained on percentage of PR levels, no significant difference was found in percentage of ER levels between the control (68.2 ± 7.3%) and MPA (64.2 ± 8.0%) groups.

Influence of Medroxyprogesterone Acetate Treatment on Estrogen Receptor and Progesterone Receptor Distribution

Percentages of nuclear receptors to total receptors are shown in Table 2. Percentages of nuclear ER were constant between the drill biopsy and surgical specimens, both in the control and MPA groups. Percentages of nuclear PR were also constant between the drill biopsy and surgical specimens in the control group; however, those were significantly higher in the surgical specimens than in the drill biopsy specimens in the MPA group. These results demonstrate that MPA treatment confers no influence on ER distribution but increases percentages of nuclear PR due to nuclear translocation.

TABLE 1. Changes in Estrogen Receptor and Progesterone Receptor Levels Between Drill Biopsy and Surgical Specimens in Control and Medroxyprogesterone Acetate Groups

Group	ER	PR
Control group	68.2 ± 7.3*	60.7 ± 8.4
MPA group	64.2 ± 8.0	23.3 ± 7.6†

ER: estrogen receptor; PR: progesterone receptor; MPA: medroxyprogesterone acetate.

* Numbers in this table represent the percentage of the receptor levels in the surgical specimens, taking the receptor levels of the drill biopsy specimens as 100%.

† $P < 0.01$ when compared with the percentage of the PR value in the control group.

Discussion

Recently, Teicher *et al.* reported that ER and PR levels of surgical specimens obtained after mastectomy were

TABLE 2. Changes in Nuclear and Cytosolic Distributions of Estrogen Receptor and Progesterone Receptor Between Drill Biopsy and Surgical Specimens in Control and Medroxyprogesterone Acetate Groups

	ER		PR	
Group	Drill biopsy specimen	Surgical specimen	Drill biopsy specimen	Surgical specimen
Control group	29.4 ± 2.9*	26.9 ± 2.2	19.7 ± 4.2	20.0 ± 2.2
MPA group	30.5 ± 3.5	27.9 ± 3.3	16.2 ± 2.8	38.3 ± 5.6†

ER: estrogen receptors; PR: progesterone receptors; MPA: medroxyprogesterone acetate.

* Nos. in this table represent the percentage of nuclear receptor levels to total receptor levels.

† $P < 0.01$ when compared with the percentage of the PR value of the drill biopsy specimen in the MPA group.

significantly lower than those of incisional biopsy specimens obtained before mastectomy, probably due to the damage occurring during surgery mainly by devascularization.[14] We have confirmed this phenomenon by comparing the receptor values of the drill biopsy specimens performed just before mastectomy with those of the surgical specimens,[10] *i.e.,* the receptor values in the surgical specimens become lower than those in the drill biopsy specimens due to the damages during the surgery. Therefore, in order to correctly evaluate the influence of MPA treatment on ER and PR levels, comparison should be made between the MPA-treated and MPA-nontreated groups and the degree of decrease in ER and PR levels should be examined.

This is why we prepared the control group, whereas the control group is usually omitted in other studies when taking the samples before and after treatment from the same patient because the value obtained before treatment usually can serve as a good control. However, this is not the case in ER and PR determination. In fact, ER and PR levels of the surgical specimens decreased to 68.2% and 60.7% of those of the drill biopsy specimens, respectively, in the control group (Table 1). This decrease is considered to be attributable to the damages conferred during mastectomy since no treatment was given between the drill biopsy and mastectomy in the control group.

It seems more advantageous to compare the results of two drill biopsies performed before and after the MPA treatment since the influence of mastectomy on the receptor values can be avoided. We think, however, it is ethically not allowed to perform the drill biopsy two times on the same patient. Currently the possibility cannot be completely neglected that repeated drill biopsies enhance the incidence of metastasis. That is why we employed the alternative, but valid, method mentioned above.

We used EIA for the determination of ER and PR instead of the conventional tritiated-ligand binding assay, since EIA can be performed accurately on such a small sample obtained from drill biopsy[10] and, moreover, EIA can detect the receptors whether or not they are occupied with corresponding hormones and seems best suited for the assay of nuclear receptors which are mostly in the occupied form.[11]

Serum MPA levels were assayed in eight patients on the day of surgery and the mean MPA level was as high as 145 ± 20 (SE) ng/ml. Medroxyprogesterone acetate treatment significantly decreased the total PR levels (Table 1) and increased the percentages of nuclear PR (Table 2). These results, taken together, demonstrate that 7-day treatment of MPA at 1200 mg/day is enough to raise the serum MPA level above 100 ng/ml and surely affects the breast cancer cells, resulting in nuclear translocation and processing of PR.[1]

Unlike the results reported on the endometrial cancer, MPA neither down regulated ER nor affected the ER distribution. Taken together with the report of Teulings *et al.*[8] that progestins do not increase E_2DH activity, our results strongly demonstrate that MPA does not elicit its antiestrogenic effects on breast cancer in the manner reported on the endometrial cancer.

Recently, Hissom and Moore have reported an interesting result that progestins do slightly but significantly stimulate the growth of human breast cancer cells (T47D) in the phenol red-free medium[15] and Robinson and Jordan have reported that progestins enhance the growth of DMBA-induced rat mammary tumors.[16] Moreover, it is well established that progestins stimulate the growth of normal breast glands in synergism with estrogens. These results seem to challenge the conventional idea, extrapolated from the results on the endometrium and endometrial cancer, that MPA directly inhibits the growth of breast cancer.

Broad receptor specificity of MPA, however, makes the understanding of its action mechanism difficult and we cannot rule out the possibility that MPA directly inhibits the growth of breast cancer, not through PR, but through glucocorticoid receptor (GR) and androgen receptor (AR). In the high-dose MPA treatment, three receptor systems (PR, AR, and GR) are considered to be involved. Currently it is unknown how these receptor systems interact to modulate the tumor growth.

In conclusion, we believe that the direct growth inhibitory effect through PR, if any, is not a major pathway through which MPA exerts its antitumor effects on human breast cancer and that the major pathway of MPA action is to suppress the adrenal function in postmenopausal women. An inverse relationship between plasma cortisol levels and response rates appears to warrant our thesis.[4]

REFERENCES

1. Horwitz KB, Wei LL, Sedladek SM, D'Arville CN. Progestin action and progesterone receptor structure in human breast cancer: A review. *Recent Prog Horm Res* 1985; 41:249–316.

2. Hellman L, Yoshida K, Zumoff B, Levin J, Kream J, Fukushima DK. The effect of medroxyprogesterone acetate on the pituitary–adrenal axis. *J Clin Endocrinol Metab* 1976; 42:912–917.

3. Blossey HC, Wander HE, Koebberling J, Nagel GA. Pharmacokinetic and pharmacodynamic basis for the treatment of metastatic breast cancer with high-dose medroxyprogesterone acetate. *Cancer* 1984; 54: 1208–1215.

4. Wander HE, Blossey HC, Koebberling J, Nagel GA. High dose medroxyprogesterone acetate in metastatic breast cancer: Correlation between tumor response and endocrine parameters. *Klin Wochenschr* 1983; 61:553–560.

5. Tseng L, Gurpide E. Effects of progestins on estradiol receptor levels in human endometrium. *J Clin Endocrinol Metab* 1975; 74:402–404.

6. Gurpide E, Tseng L, Gusberg SB. Estrogen metabolism in normal and neoplastic endometrium. *Am J Obstet Gynecol* 1977; 129:809–816.

7. Pollow K, Boquoi E, Lubbert H, Pollow B. Effect of gestagen therapy

upon 17-hydroxysteroid dehydrogenase in human endometrial adenocarcinoma. *J Endocrinol* 1975; 67:131–132.

8. Teulings FAG, Gilse HA, Henkelman MS, Portengen H, Alexieva-Figusch J. *Cancer Res* 1980; 40:2557–2561.

9. Lundgren S, Kvinnsland S, Varhaug JE, Utaaker E. The influence of progestins on receptor levels in breast cancer metastasis. *Anticancer Res* 1987; 7:119–124.

10. Noguchi S, Miyauchi K, Inaji H, Imaoka S, Koyama H. Enzyme immunoassay for estrogen and progesterone receptors in drill biopsy specimens from breast cancer. *Eur J Cancer Clin Oncol* 1989; 25:809–814.

11. Noguchi S, Miyauchi K, Imaoka S, Koyama H, Iwanaga T. Comparison of enzyme immunoassay with dextran-coated charcoal method in the determination of progesterone receptor in breast cancer cytosols. *Eur J Cancer Clin Oncol* 1988; 24:1715–1719.

12. Burton KH. A study of the conditions and mechanism of diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* 1956; 62:315–323.

13. Lowry OH, Rosebrough NJ, Farr AK, Randwall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265–275.

14. Teicher I, Tinker MA, Auguste LJ, Laufer H, Stein TA, Wise L. Effect of operative devascularization on estrogen and progesterone receptor levels in breast cancer specimens. *Surgery* 1985; 98:784–791.

15. Hissom JR, Moore MR. Progestin effects on growth in the human breast cancer cell line T-47D. *Bichem Biophy Res Commun* 1987; 145: 706–711.

16. Robinson SR, Jordan VC. Reversal of the antitumor effects of tamoxifen by progesterone in the 7,12-dimethylbenzanthracence-induced rat mammary carcinoma model. *Cancer Res* 1987; 47:5386–5390.