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Original Paper

Predictive Value of Serum Medroxyprogesterone Acetate Concentration for Response in Advanced or Recurrent Breast Cancer

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Medroxyprogesterone acetate (MPA) is one of the most commonly prescribed drugs for endocrine therapy of metastatic breast cancer. In this study, the serum MPA concentration was measured by high-performance liquid chromatography (HPLC) and evaluated for its usefulness in predicting the response in 79 cases of advanced or recurrent breast cancers. Overall, 29 patients (37%) achieved an objective response. The response rate correlated significantly with the oestrogen receptor (ER) status ($P = 0.03$), proliferative activity determined by DNA polymerase α ($P = 0.04$), the disease-free interval (DFI) ($P = 0.05$) and the serum MPA concentration ($P < 0.001$). Patients with ER-positive tumours, lower proliferative activity, a longer (DFI) or a higher serum MPA concentration responded more frequently. The mean serum MPA concentration in the responders with ER-positive tumours ($P = 0.01$) or tumours with a lower proliferative activity ($P = 0.008$) were significantly lower than in cases with ER-negative tumours or tumours with a higher proliferative activity, respectively. Cases with soft tissue metastases showed responses at significantly lower MPA concentrations ($P = 0.003$) than those with bone or visceral metastases. Furthermore, there was a dramatic decrease in the MPA concentration when a responder with a high concentration became unresponsive to the therapy. Thus, the serum MPA concentration is a determining factor for the response to treatment. © 1997 Elsevier Science Ltd.

Key words: breast cancer, medroxyprogesterone acetate

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INTRODUCTION

AT PRESENT, medroxyprogesterone acetate (MPA) is widely used as a second- or third-line drug in endocrine therapy for advanced breast cancer. The overall clinical response rate to high doses of MPA averages 40% in unselected breast cancer patients [1–3]. However, in some cases, MPA caused various adverse effects, including thromboembolic disease. Therefore, it is desirable to select patients likely to benefit from MPA therapy. Various mechanisms have been proposed to explain the efficacy of MPA treatment, but the action of MPA on human breast cancer remains poorly understood.

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It has not been possible to define a clear dose–response correlation because the same dose of MPA can generate vastly different drug serum levels [4, 5]. We recently found that there are significant relationships between the serum MPA level and the response to the therapy, improvement in the performance status and survival, although patients received different doses of MPA and there was a high inter-patient variability in the serum level even with the same dose of MPA [6]. Moreover, patients with both oestrogen (ER) and progesterone receptor (PgR)-positive tumours responded to the therapy. In order to understand the action of MPA, it is important to examine the difference in its mode of action between ER-positive and ER-negative tumours.

In this study, we examined the relationship between the serum MPA concentration and the biological characteristics

of advanced or recurrent breast cancer, and also the change in the serum level during progression of the disease.

PATIENTS AND METHODS

Patients

A total of 79 patients with recurrent or advanced breast cancer were entered in this trial between April 1992 and December 1995. The mean age was 50 years (range, 28–92), and 52 patients were postmenopausal. The patients were randomly assigned to receive MPA orally at approximately 600 mg/day (49 cases) or 800 mg/day (19 cases). In some older cases (5 cases) with small physiques, only 400 mg/day was given, and in some cases who did not respond to 600 or 800 mg/day of MPA, the dosage was increased to 1200 mg/day (6 cases). The number of drug doses per day depended on the dosage (e.g. two for 400 or 800 mg/day; three for 600 or 1200 mg/day). We regularly questioned the outpatients regarding their drug intake, while we controlled the drug intake of the hospitalised patients. All patients had a life expectancy of 2 months or more at the time of enrolment in this study.

As shown in Table 1, 41 patients had previously received systemic treatment, while 10 patients had been treated by radiotherapy but the irradiated lesions were different from the lesions studied in this trial. Regarding the most common sites of metastasis, 17 patients had soft tissue, 28 had bone and 34 had visceral metastases.

Evaluation of response

The objective response was assessed at monthly intervals in accordance with the criteria of the Japanese Breast Cancer Society (1992). A complete response (CR) referred to disappearance of all measurable lesions for at least 4 weeks; a partial response (PR) corresponded to more than a 50% decrease in measurable lesions for at least 4 weeks; and no change (NC) referred to patients whose lesions decreased by less than 50% or increased by less than 25% for at least 8 weeks. The records of all patients in this study were assessed by extramural physicians. The duration of a response was defined as the length of time from the onset of the response until progression of disease became evident.

Table 1. Pretreatment characteristics of evaluable patients

No. of patients	79
Age (years)	50 (28–92)
DFI (months)	28 (2–147)
Previous therapy	
Tamoxifen alone	8
Chemotherapy with TAM	17
Chemotherapy alone	16
Radiotherapy alone	10
Time from first recurrence	Median (range)
to start of MPA (months)	4 (0–131)
Time from start of MPA	Median (range)
to death (months) ($n = 32$)	10.0 (2.0–51.0)
Performance status	
0	$n = 11$
1	$n = 16$
2	$n = 18$
3	$n = 15$
4	$n = 20$
Dominant site of metastasis	
Soft tissue	17
Bone	28
Viscera	34

DFI, disease-free interval.

MPA and receptor assay

The serum MPA levels were determined by means of a specific HPLC (high-performance liquid chromatography) method, as described previously [6]. All blood samples were collected from 8 to 10 a.m., before MPA administration, and stored at −20°C until analysed. Most samples were obtained every 3–4 months, beginning at least 2 weeks after the start of therapy. Concentrations determined close to (mostly within 1 month) the time of evaluation of the treatment response were subjected to analysis of the relationship between the serum MPA concentration and the therapeutic efficacy, and the mean time from the start of MPA therapy to sampling was 3 months. Blood samples were not taken prior to entry into this study. The MPA serum level was below 5 ng/ml by this assay in patients who were not administered MPA.

ER was assayed in samples of the primary tumour by EIA (enzyme immunoassay) using a monoclonal antibody (Abbott ER-EIA monoclonal). ER was considered positive if ≥14 fmol/mg protein.

DNA polymerase α immunostaining

DNA polymerase α is a key enzyme for DNA replication in the nuclei of all proliferating eukaryotic cells [7], and it is associated with cell proliferation, independent of the cell cycle [8]. DNA polymerase α immunoreactivity was demonstrated using a peroxidase–antiperoxidase (PAP) kit, which employed a mouse IgG monoclonal antibody, CL22-4-42B (Medical Biological Laboratory, Tokyo, Japan) [9]. The proliferative activity of each tumour was classified semi-quantitatively into three groups according to the percentage of positive nuclei: <20%, ≥20 <50%, and ≥50%.

Statistical analysis

Fisher's exact test was used to test for associations between the objective response and clinicopathological variables (daily dosage, menopausal status, dominant site of metastasis, previous therapy, disease-free interval (DFI), histological type, hormone receptor and DNA polymerase α), as well as those between the objective response and the serum MPA concentration reported in Tables 2–5. The Wilcoxon two-sample test and the Kruskal–Wallis test were used to assess significant differences in the serum MPA concentration among groups. In our previous report [6], we discussed that there was a significant difference between the objective response and the serum MPA concentration stratified 25 and 55 ng/ml. The same stratification was used for this study. All P values presented are two-sided and P values less than 5% were judged as statistically significant. Statistical analyses were carried out using the Statistical Analysis System (SAS).

RESULTS

Response to MPA therapy

Of the 79 patients included in this study, three (4%) were rated as CR and 26 (33%) as PR. The overall response rate was 37%.

Table 2. Response to MPA therapy and clinicopathological variables

Variable	CR + PR (%)	NC	PD	Fisher's exact test	Total
Total	29 (37)	28	22		79
Daily dosage of MPA (mg)					
400	2 (40)	1	2		5
600	12 (24)	22	15		49
800	11 (58)	3	5	$P = 0.05$	19
1200	4 (67)	2	0		6
Menopausal status					
Pre-	11 (41)	7	9	NS	27
Post-	18 (35)	21	13	($P = 0.48$)	52
Dominant site of metastasis					
Soft tissue	5 (29)	6	6	NS	17
Bone	11 (39)	12	5	($P = 0.61$)	28
Viscera	13 (38)	10	11		34
Previous therapy					
No	14 (37)	14	10	NS	38
Yes	15 (37)	14	12	($P = 0.96$)	41
DFI					
<2 year	7 (22)	13	12	$P = 0.05$	32
⩾2 years	20 (49)	13	8		41
Histological type					
Papillotubular carcinoma	2 (20)	6	2		10
Solid-tubular carcinoma	7 (39)	3	8	NS	18
Scirrhous carcinoma	14 (36)	16	9	($P = 0.16$)	39
Other	2	1	1		
Unknown	4	1	2		
Hormone receptor					
ER+	16 (57)	6	6	$P = 0.03$	28
ER–	11 (26)	19	13		43
DNA polymerase α					
<20%	13 (52)	7	5	$P = 0.04$	25
⩾20%	4 (17)	9	10		23

For some categories, data were missing so the total was less than 79. CR, complete response; PR, partial response; NC, no change; PD, progressive disease; NS, not significant; DFI, disease-free interval.

Table 2 shows the relationship between the response to MPA and the clinicopathological variables. The response rate did not increase with the daily dose of MPA, but 4 of the 6 patients who had not responded to the 600 or 800 mg/day of MPA achieved a response when administered 1200 mg/day. The response rate did not correlate with the menopausal status, dominant site of metastasis, previous therapy or histological type. Patients with ER-positive tumours responded to the therapy more frequently than patients with negative tumours ($P = 0.03$), and the response rate of tumours with higher proliferative activity (DNA polymerase $\alpha \geq 20\%$) was significantly lower ($P = 0.04$) than those with lower proliferative activity (DNA polymerase $\alpha < 20\%$).

As shown in Table 3, there was a significant relationship ($P < 0.001$) between the MPA serum level and clinical response. Objective responses were observed in 16 of 18 (89%) patients with a serum MPA level above 55 ng/ml, but in only 14% of patients with a low concentration (< 25 ng/ml). Moreover, the serum MPA concentration of the patients showing CR or PR (60.1 ± 31.3 ng/ml) to the therapy was significantly higher than in those with NC (33.8 ± 20.2 ng/ml; progressive disease (PD) $P < 0.001$) or PD (23.4 ± 9.6 ng/ml; ($P < 0.001$). Moreover, Table 4

Table 3. MPA serum level and response to therapy

Serum level	CR + PR No. (%)	NC No. (%)	PD No. (%)	Fisher's exact test*
<25 ng/ml ($n = 28$)	4 (14)	11 (39)	13 (46)	
25–< 55 ng/ml ($n = 33$)	9 (27)	15 (45)	9 (27)	*$P < 0.001$
⩾55 ng/ml ($n = 18$)	16 (89)	2 (11)	0	
Mean ± S.D.	60.1 ± 31.3	33.8 ± 20.2	23.4 ± 9.6	
†Wilcoxon two-sample test		†$P < 0.001$	†$P = 0.03$	
		†$P < 0.001$		

For abbreviations, see legend to Table 2.

Table 4. Serum MPA level and response to therapy as a function of menopausal or ER status

Factor/Serum level (ng/ml)	CR + PR (%)	NC	PD	Fisher's exact test	Total
Menopausal status					
Pre-					
<25	3 (27.2)	3	5		11
25– < 55	2 (20.0)	4	4	$P = 0.03$	10
⩾55	6 (100)	0	0		6
Post-					
<25	1 (5.8)	7	9		17
25– < 55	7 (30.4)	11	5	$P < 0.001$	23
⩾55	10 (83.3)	2	0		12
ER status					
Positive					
<25	3 (30)	4	3	NS	10
25– < 55	7 (58.3)	2	3	$P = 0.11$	12
⩾55	6 (100)	0	0		6
Negative					
<25	0	6	8		14
25– < 55	2 (11.1)	11	5	$P < 0.001$	18
⩾55	9 (81.3)	2	0		11

For abbreviations, see legend to Table 2.

shows the relationship between the MPA serum level and response to the therapy as a function of the menopausal status or ER status. There was a significant relationship between the serum MPA level and the response rate regardless of the menopausal status (Pre, $P = 0.03$; Post, $P < 0.001$). However, this significant relationship was seen only in patients with ER-negative tumours ($P < 0.001$). These findings showed that lower serum MPA levels could yield a response in patients with ER-positive tumours.

MPA serum levels in responders to the therapy

Table 5 shows the differences in the serum MPA levels in responders to the therapy according to the ER status, DNA polymerase α activity and dominant site of metastasis. The mean serum MPA levels of patients with ER-positive tumours ($P = 0.01$) or with a low DNA polymerase α activity ($P = 0.008$) were significantly lower than in those with tumours which were ER-negative or showed a high DNA polymerase α activity. There was also a significant relationship between the mean serum MPA level and the dominant site of metastasis ($P = 0.003$). Patients with soft tissue metastases achieved responses at significantly lower MPA levels than those with bone or visceral metastases. Thus, the serum MPA level needed to achieve a response differed as a function of the tumour.

Change in serum MPA level

The change in the serum MPA level was examined in responders (PR or CR) who became unresponsive to the therapy. As shown in Figure 1, the serum MPA level (mean ± S.D. = 73.8 ± 35.5 ng/ml) during CR or PR (12 cases) was significantly higher ($P = 0.00015$) that in subsequent PD (mean ± S.D. = 17.6 ± 13.8 ng/ml). However, the MPA concentrations in patients (10 cases) whose response lasted longer than 6 months (CR, PR or NC) were stable throughout that period.

DISCUSSION

MPA is one of the most widely used compounds for endocrine therapy of advanced breast cancer, and it is generally used for patients who have relapsed after undergoing other endocrine therapeutic modalities. In this study, we confirmed our earlier finding [6] that serum MPA concentration correlates significantly with the clinical response to the therapy in advanced or recurrent breast cancer. To evaluate further the clinical usefulness of the serum MPA concentration as a predictor of a response, we examined the differences in the serum MPA concentrations among responders and the change in the concentration as a function of the clinical response.

There was a significant relationship between the serum MPA level and the response in patients with ER-negative

Table 5. MPA serum level according to ER, DNA polymerase α or dominant site of metastasis in patient with CR or PR

Serum MAA	ER		DNA polymerase α		Dominant site		
Level (ng/ml)	+	–	<20%	⩾20%	Soft tissue	Bone	Viscera
<25	3	0	2	0	3	1	0
25– < 55	7	2	5	0	2	5	2
⩾55	6	9	6	4	0	5	11
Total	16	11	13	4	5	11	13
Mean ± S.D.	48.1 ± 24.7	77.8 ± 30.9	49.9 ± 22.8	92.7 ± 15.9	26.6 ± 10.4	53.8 ± 25.3	78.3 ± 29.2
†Wilcoxon two sample test	†$P = 0.01$		†$P = 0.008$		‡$P = 0.003$		
‡Kruskal–Wallis test							

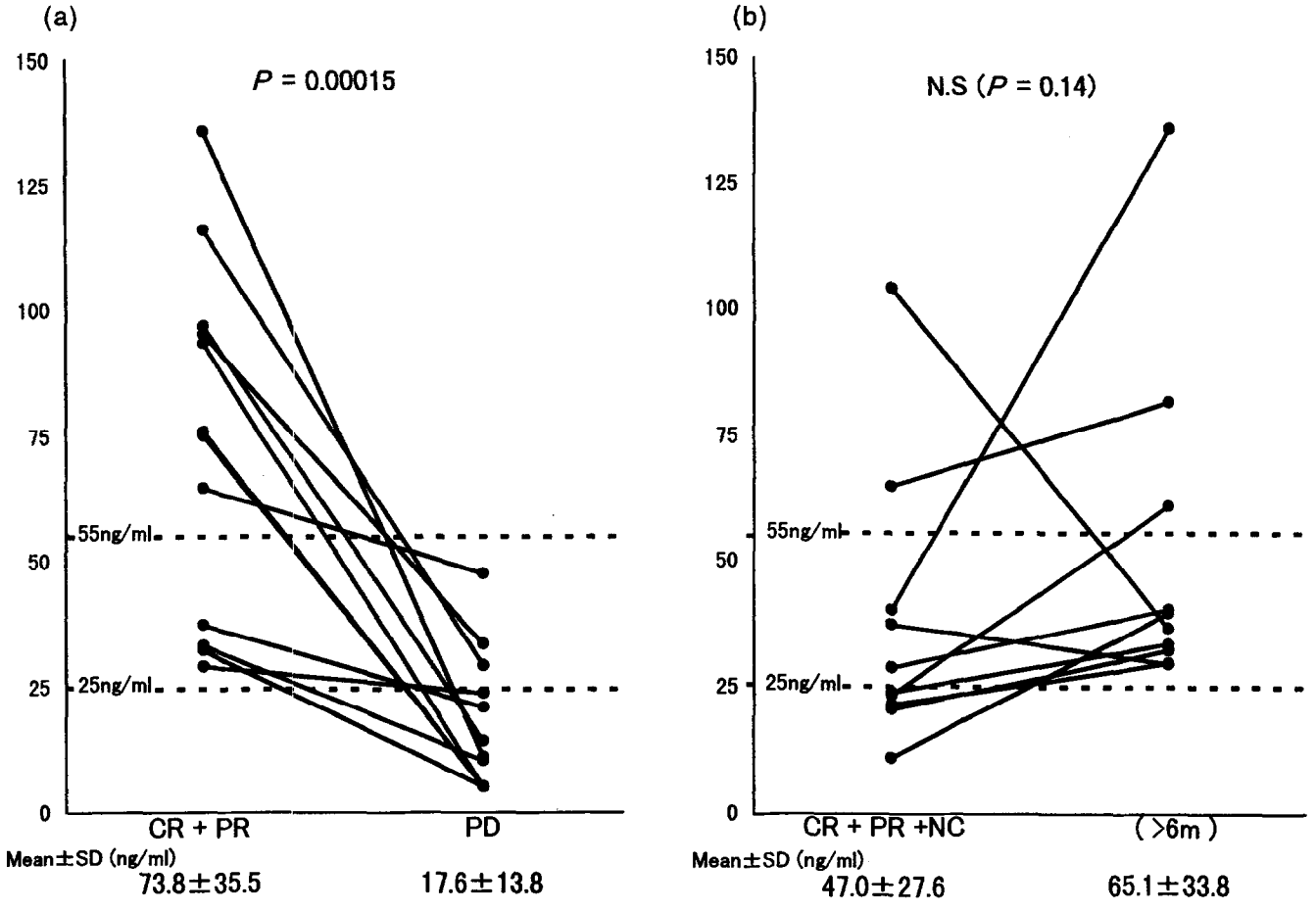

Figure 1. Change in serum MPA level as function of response to MPA therapy. (a) Dramatic decreases in the serum MPA level were seen in responders (12 cases) who became unresponsive to the treatment. (b) The serum level was generally stable in patients (10 cases) whose response lasted longer than 6 months.

tumours but not in those with ER-positive tumours. These findings showed that a lower serum MPA level could yield a response in patients with ER-positive tumours. There were significant differences in the serum MPA concentration between ER-positive and ER-negative tumours, and also between tumours with lower and higher proliferative activity in the responders to MPA therapy. Tumours which were ER-positive or showed a lower proliferative activity responded more frequently to MPA therapy at a significantly lower serum MPA concentration. Patients with ER-positive tumours or tumours with a lower proliferative activity obtained more benefit, whereas patients with ER-negative tumours or tumours with a higher proliferative activity were able to respond to the therapy only when the serum MPA concentration was high. We previously found [6] a significant relationship between the serum MPA concentration and the dose per body surface area (mg/cm^2) in cases with CR, PR or NC following therapy, and the measured level was lower than predicted when the patient received little benefit from the MPA therapy. In patients with PD, the serum MPA level did not rise to an adequate level to achieve an effect. Therefore, most patients with ER-negative tumours, which seldom respond to MPA therapy, might not have an adequate MPA serum level. These findings suggest that the optimal serum MPA concentration might depend on individual biological characteristics of tumours. Li and associates [10] found that a low and constant MPA blood level (3.14 ± 0.32 ng/ml) caused growth inhibition of 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced tumours. DMBA tumours are considered to be receptor-positive. Poulin and associates [11] found that the inhibitory effect of MPA on the growth of cultured human breast cancer cells (ZR-75-1), which were ER-, PgR- and AR-positive, was mainly due to the androgenic properties of the compound, exerted at nanomolar concentrations. Their findings are in agreement with our data. In receptor-positive tumours, a lower serum level of MPA is likely to achieve therapeutic efficacy. However, in receptor-negative tumours, a higher serum level of MPA may still exert a beneficial effect but by a different mechanism.

Furthermore, we demonstrated that the serum MPA concentrations of responders decreased dramatically when they became unresponsive to the therapy, whereas the concentration remained almost constant during maintenance of the same response. This finding suggests that the response status depends on the serum MPA concentration, which might be affected by a change in the enzymatic conversion capacity of the patient. Regarding the level of MPA metabolites in the serum, Sturm and associates [12] identified 16 kinds of MPA metabolites in serum by HPLC. It was demonstrated that the measured metabolite levels showed a dramatic increase in a non-responder to MPA although the serum MPA level was very low, with the hydrated products and glucuronides appearing to be predominant. Thus, indi-

vidual differences in steady-state MPA levels may be assumed to be due to different enzymatic conversion capacities of the patients.

Various mechanisms for the efficacy of MPA have been proposed. Inhibition of ovarian oestrogen synthesis may achieve indirect inhibition of tumour growth by reduction of follicle stimulating hormone (FSH) and luteinising hormone (LH) secretion [13]. MPA may suppress adrenal function and reduce the plasma cortisol level [14]. Direct inhibition of the proliferation of human mammary cancer cell lines by MPA has been discussed in relation to ER, PgR, AR and glucocorticoid receptor [15–17]. Elizalde and associates [18] suggested that one of the mechanisms was inhibition of the expression of transforming growth factor-β_1. Using a rabbit corneal system for assaying angiogenesis, several investigators [19, 20] showed that MPA inhibited angiogenesis. Furthermore, Ashino-Fuse and associates [21] found that MPA decreased the activity of plasminogen activator. Thus, MPA shows various actions, and its role in breast cancer remains controversial.

In conclusion, the serum MPA concentration is one of the most important factors influencing the efficacy of MPA treatment for advanced or recurrent breast cancer. A high serum MPA concentration enhances the possibility of a response to MPA therapy, but in ER-positive tumours or tumours with lower proliferative activity, a low serum MPA level can have an effect. Moreover, a decrease in the serum MPA level in responders may accompany loss of the drug's effects. Thus the serum MPA concentration is a determining factor for response to treatment.

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