Jpn. J. Cancer Res. 85, 542–549, May 1994

Modulation of Adriamycin Resistance in Human Breast Carcinoma MCF-7 Cells *in vitro* and *in vivo* by Medroxyprogesterone Acetate

Hiroyuki Ishida,[1] Masami Okabe,[1] Katsushige Gomi,[1,5] Ryuya Horiuchi,[2] Koji Mikami,[4] Mikihiko Naito[4] and Takashi Tsuruo[3,4]

[1]*Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Shimotogari 1188, Nagaizumi-cho, Sunto-gun, Shizuoka 411,* [2]*Department of Pharmacy, School of Medicine, Gunma University, Showa-machi 3-39-22, Maebashi, Gunma 371,* [3]*Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Kami-Ikebukuro 1-37-1, Toshima-ku, Tokyo 170 and* [4]*Institute of Molecular and Cellular Biosciences, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113*

The combination effect of adriamycin (ADM) and medroxyprogesterone acetate (MPA) was examined *in vitro* against human breast carcinoma MCF-7 and its ADM-resistant line (MCF-7/ADM). MCF-7 cells, which are positive for estrogen receptors, progesterone receptors and high-affinity MPA-binding activity, were more susceptible to the growth-inhibitory activity of ADM or MPA than MCF-7/ADM cells. A combination effect of ADM and MPA was observed against MCF-7/ADM cells, which are negative for steroid receptors, and furthermore against human nasopharynx carcinoma KB and its ADM-resistant line KB-A1. This combination effect of ADM and MPA against MCF-7/ADM cells was demonstrated to be synergistic by using the median effect plot method. The activity of MPA was almost equivalent to that of chlormadinone acetate or tamoxifen, greater than that of progesterone, and less than that of verapamil. The accumulation of ADM in MCF-7/ADM cells was enhanced by treatment with 10 μM MPA as well as 10 μM verapamil. The efflux of accumulated ADM from MCF-7/ADM cells was also partially inhibited by treatment with MPA or verapamil. MPA augmented the growth-inhibitory activity of ADM against MCF-7/ADM tumors inoculated into nude mice, although statistical significance was not observed. It is suggested that the clinical advantage of the combination of MPA with ADM against advanced breast cancers may be partly explained by the modulation of ADM resistance by MPA.

Key words: Adriamycin — Medroxyprogesterone acetate — Verapamil — Multidrug resistance

The effectiveness of hormonal therapy with high-dose MPA,[6] a synthetic progesterone derivative, has been established clinically against advanced breast cancer[1,2] and endometrial cancer.[3,4] Furthermore, MPA can augment the efficacy of first-line combination chemotherapy of advanced breast and endometrial cancer including cyclophosphamide, ADM or 5-fluorouracil.[5,6] We have demonstrated that the combination of MPA and 5-fluorouracil had additive growth-inhibitory activity against cultured human breast carcinoma MCF-7 cells and stomach carcinoma cells *in vitro*, while MPA reduced the lethal toxicity and bone marrow toxicity of 5-fluorouracil in mice,[7,8] indicating the rationale of this combination. Concerning the combination of ADM and MPA, the pretreatment of MCF-7 cells with MPA was reported to augment the growth-inhibitory activity of ADM additively.[9]

The emergence of drug-resistant tumor cells during treatment is one of the major problems in cancer chemotherapy. When tumor cells acquire resistance to *Vinca* alkaloids or anthracyclines, they usually show cross-resistance to other antitumor agents, i.e., so-called multidrug resistance.[10,11] The mechanism of multidrug resistance is an enhanced efflux of drugs from tumor cells and subsequent reduction of intracellular drug concentration.[12,13] P-Glycoprotein is involved in this efflux, and its overexpression has been reported in many multidrug-resistant tumor cells.[14,15] Various compounds including verapamil have been found to overcome multidrug resistance.[16,17] Another endocrine drug, tamoxifen, is also reported to modulate multidrug resistance *in vitro* in ADM-resistant murine P388 leukemia[18] and human breast carcinoma MCF-7 cells.[19] Various steroid hormones also inhibit the binding of *Vinca* alkaloids to P-glycoprotein, and progesterone has the highest affinity to P-glycoprotein among them.[20,21] Although MPA exhibits higher growth-inhibitory activity against hormone-dependent breast cancer cell lines than progesterone, its effect on multidrug resistance has not been reported. The present study was carried out in order to investigate the

[5] To whom correspondence should be addressed.

[6] Abbreviations: MPA, medroxyprogesterone acetate; ADM, adriamycin; CI, combination index; IC_{50}, concentration required for 50% growth inhibition; PBS (−), Dulbecco's phosphate-buffered saline (Ca^{2+}-, Mg^{2+}-free); TAM, tamoxifen.

in vitro combination effect of ADM and MPA in terms of ADM resistance.

MATERIALS AND METHODS

Chemicals ADM (Kyowa Hakko Kogyo Co., Tokyo), was dissolved in sterile distilled water. MPA (Kyowa Hakko Kogyo Co.), progesterone (Nacalai Tesque Co., Kyoto), hydrocortisone, progesterone, chlormadinone acetate and TAM (Sigma Chemical Co., St. Louis, MO), [^{3}H]MPA (44.9 Ci/mmol, New England Nuclear, Boston, MA), and [^{3}H]17β-estradiol (110 Ci/mmol, Amersham International, Buckinghamshire, UK) were each dissolved in ethanol. Verapamil (Sigma Chemical Co.) was dissolved in dimethyl sulfoxide. These drug solutions were diluted with the culture medium, and the final concentration of solvent was 0.5% or less. [^{14}C]-ADM (55 mCi/mmol, New England Nuclear) was dissolved in methanol.

Tumor cells Human breast carcinoma MCF-7 and its ADM-resistant line (MCF-7/ADM, gift from Dr. K. H. Cowan, National Cancer Institute, USA)[22,23] were passaged in RPMI-1640 medium (Grand Island Biological Co., Grand Island, NY) containing 10% fetal bovine serum (Grand Island Biological Co.), 100 IU penicillin, 100 μg streptomycin/ml (Grand Island Biological Co.) and 10 n*M* 17β-estradiol (Sigma Chemical Co.) at 37°C in a humidified atmosphere containing 5% CO_2 in air. Human nasopharynx carcinoma KB and its ADM-resistant line (KB-A1, gift from Dr. K. Ueda, Kyoto University)[15] were passaged in Dulbecco's minimal essential medium (Nissui Pharmaceutical Co., Tokyo) containing 10% fetal bovine serum.

Cell growth-inhibitory activity The cells (2.5×10^3/well) were precultured for 24 h in 24-well multidishes (Nunc, Roskilde, Denmark) with 0.75 ml of Eagle's minimal essential medium (phenol red-free, Nissui Pharmaceutical Co.) containing 5% calf serum (HyClone, Logan, UT), 110 μg sodium pyruvate/ml, 1% (v/v) non-essential amino acids (Flow Lab., McLean, VA) and 1 n*M* 17β-estradiol (hereafter designated as culture medium) in each well, and treated with various compounds for 144 h. The growth-inhibitory activity of compounds was evaluated by counting cell number using a Micro-cell counter (Toa Medical Electronics Co., Hyogo).

Analysis of combination effect The combination effect of ADM with each compound was analyzed in terms of expected IC_{50} value and combination index, respectively. The expected IC_{50} value for ADM used in combination with a certain concentration of each compound was obtained from the expected growth percentage calculated by use of the following formula[24]:

Expected growth (%) = [growth (%) with ADM] × [growth (%) with each compound] × 1/100.

When the actual IC_{50} value of ADM in combination with a certain concentration of a compound is equal to the expected IC_{50} value, the interaction is considered to be additive; when the actual IC_{50} value is less than expected IC_{50} value, synergism is indicated, and when the actual IC_{50} value is over the expected IC_{50} value, antagonism is indicated.

For more precise analysis of combination effect, the median effect plot method established by Chou and Talalay was used.[25] A combination index (CI) was determined by drawing a least-squares regression line on a computer graphic system. When CI is 1, the interaction is considered to be additive; when CI is less than 1, synergism is indicated, and when CI is over 1, antagonism is indicated.

Binding of steroids to the cells The cells (1×10^5/well) were precultured in 24-well multidishes (Nunc) containing 0.5 ml of the culture medium in each well at 37°C for 24 h. For the assay of 17β-estradiol binding, the culture medium was deprived of it. The cells were then treated with [^{3}H]MPA (10 n*M*) or [^{3}H]17β-estradiol (10 n*M*) with various concentrations of unlabeled MPA or 17β-estradiol, respectively, and incubated at 37°C for 60 min. The cells were treated with PBS(−) containing 10% (v/v) glycerol and 0.5% (w/v) bovine serum albumin at 4°C for 30 min, and washed twice with the above buffer solution. The cells were lysed with 1 *N* NaOH solution at 37°C overnight, and their radioactivity was measured with a liquid scintillation counter.

Uptake and efflux of labeled ADMs The cells (1×10^6/well) were precultured in 6-well plates (Nunc) containing 2 ml of the culture medium in each well at 37°C for 24 h. The cells were then treated with 0.1 ml of [^{14}C]ADM and 0.1 ml of each compound solution, incubated for the indicated time, and washed twice with ice-cold PBS(−). The cells were lysed with 1 *N* NaOH solution at 37°C overnight, and their radioactivity was measured. For the determination of efflux of [^{14}C]ADM, the cells (1×10^6/2 ml) precultured in 6-well plates for 24 h were treated with 0.1 ml of [^{14}C]ADM at 37°C for 60 min. Then the cells were washed twice with PBS(−) and incubated with each compound at 37°C for the indicated time. The radioactivity of cells was measured as described above.

***In vivo* antitumor activity** MCF-7/ADM cells were prepared from the *in vitro*-cultured cell line, inoculated into the flank of male 7- or 8-week-old BALB/c-*nu/nu* mice (nude mice) weighing 20–25 g (obtained from Clea Japan Inc., Tokyo), and passaged *in vivo*. To evaluate the antitumor activity, the length and width of tumors were measured twice a week, and their volume was calculated by using the following formula according to the method of the National Cancer Institute[26]:

$$\text{Tumor volume (mm}^3\text{)} = \frac{\text{Length (mm)} \times [\text{width (mm)}]^2}{2}$$

Tumor growth rate was expressed as the mean V/V_0 value, where V is the tumor volume on the day of evaluation and V_0 is that on the day of initial administration.

RESULTS

Effect of MPA on growth-inhibitory activity of ADM

We first examined the characteristics of steroid receptors of the cell lines used in this experiment, and their sensitivity to ADM or MPA (Table I). Among them, only MCF-7 cells possessed estrogen receptors and high-affinity MPA-binding activity, and showed significant sensitivity to MPA. MCF-7/ADM and KB-Al cells were confirmed to have acquired marked resistance to ADM.

The combination effect of ADM and MPA was examined in ADM-sensitive and -resistant cell lines (Table II). The actual IC_{50} values of ADM were measured at each concentration of MPA, and they were compared with the expected IC_{50} values of ADM in combination with MPA. The combination effect of ADM plus MPA against sensitive MCF-7 cells could not be analyzed due to the significant sensitivity of MCF-7 cells to MPA. Potentiation was observed by MPA at 1–10 μM in MCF-7/ADM cells, 10 μM in KB cells and 1–10 μM in KB-A1 cells, suggesting that the combination effect of ADM and MPA is synergistic in these ranges of concentration. For more precise analysis of their combination effect, the median effect plot method was applied (Fig. 2) based on the result of Fig. 1, in which dose-responses of ADM, MPA or ADM plus MPA against MCF-7/ADM cells are

Table I. Growth-inhibitory Activity of ADM and MPA on ADM-sensitive and -resistant Tumor Cells

Cell line	Estrogen receptors		MPA-binding proteins				Growth-inhibitory activity	
			Affinity				IC_{50} (μM)	
			High		Low			
	K_d (nM)	Sites/cell ($\times 10^4$)	K_d (nM)	Sites/cell ($\times 10^4$)	K_d (μM)	Sites/cell ($\times 10^4$)	ADM	MPA
MCF-7	0.18	1.6	0.51	18	1.3	850	0.014	0.044
MCF-7/ADM		ND[a]		ND	0.59	1300	0.57	26
KB		ND		ND	0.59	790	0.0031	22
KB-A1		ND		ND	0.41	670	0.19	10

a) Not detected.

Table II. Effect of MPA on Growth-inhibitory Activity of ADM against ADM-sensitive and -resistant Tumor Cells

Cell line	MPA (μM)	Inhibition by MPA (%)	IC_{50} of ADM (μM)		Potentiation[b]
			Actual	Expected[a]	
MCF-7	0	0	0.014	0.014	1.0
	0.01	45	0.0062	0.0037	0.60
	0.1	53	0.00059	ND[c]	ND
	1	57	<0.00041	ND	ND
	10	76	<0.00041	ND	ND
MCF-7/ADM	0	0	0.57	0.57	1.0
	1	4	0.47	0.53	1.1
	3	4	0.28	0.53	1.9
	10	14	0.13	0.40	3.1
KB	0	0	0.0031	0.0031	1.0
	10	8	0.0016	0.0029	1.8
KB-A1	0	0	0.19	0.19	1.0
	1	12	0.13	0.14	1.1
	3	19	0.066	0.095	1.4
	10	72	<0.031	ND	ND

a) Indication of additive effect of ADM and MPA.
b) Expected IC_{50} versus actual IC_{50} value.
c) Not determined.

shown. The CI values of the group treated with ADM plus MPA were less than 1 at high concentrations, indicating that the cell growth-inhibitory activity of this combination regimen is synergistic. This result also indicates that the results of the median effect plot method are well correlated with those of the expected value method (Table II). Therefore, further examination was performed by this expected value method.

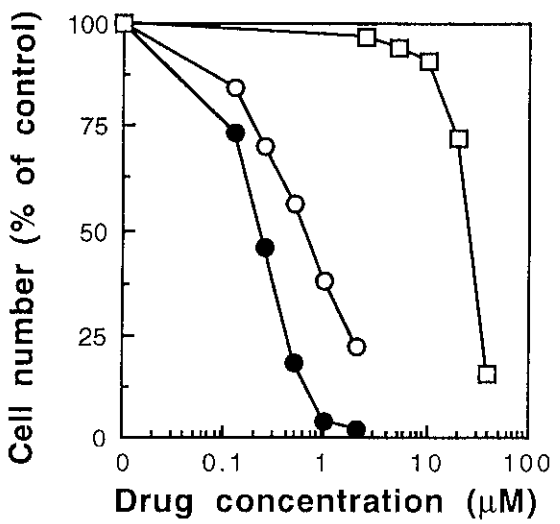

Fig. 1. Combination effect of ADM and MPA against MCF-7/ADM cells. MCF-7/ADM cells (2.5×10^3/well) were cultured on day 0, and treated with ADM (○), MPA (□) or ADM plus MPA (●) on day 1. For the combination, 2-fold dilutions of ADM (0.125–2 μM) and MPA (2.5–40 μM) were combined from the lowest concentration, and drug concentrations were expressed based on ADM concentration. The cell number was counted on day 7, and mean values are shown. The standard deviations were less than 10% of mean values.

The combination effect of ADM plus MPA was compared with those of ADM plus other steroid hormones, TAM and verapamil (Table III). Marked potentiation was observed with the combination of ADM with MPA, chlormadinone acetate, TAM or verapamil, indicating that these compounds exhibit synergistic growth-inhibitory activity in combination with ADM. Verapamil exhibited the most marked combination effect with ADM. The effect of MPA was almost equivalent to that of chlormadinone acetate or TAM, and more significant than that of progesterone. The combination effects of ADM with MPA, TAM or verapamil were observed at various concentrations of ADM (Fig. 3). MPA (10 μM) potentiated the cell growth-inhibitory activity of 0.031–

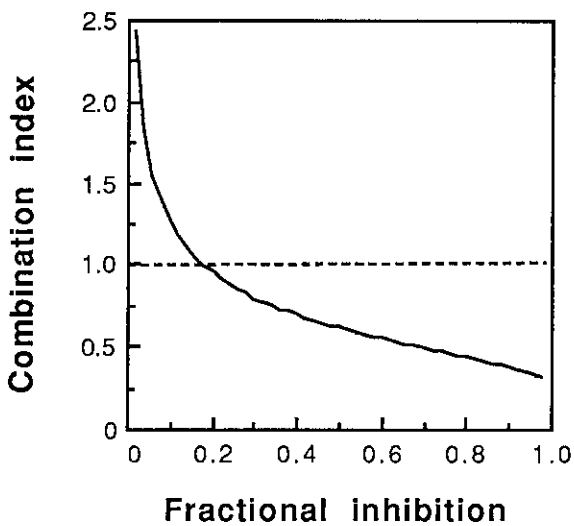

Fig. 2. Analysis of synergism of combination effect of ADM plus MPA. The results shown in Fig. 1 were analyzed by the median effect plot method.

Table III. Effect of Steroid Hormones, TAM and Verapamil on Growth-inhibitory Activity of ADM against MCF-7/ADM Cells

Modulator	Dose (μM)	Inhibition by modulator (%)	IC_{50} of ADM (μM)		Potentiation[b]
			Actual	Expected[a]	
None	0	0	0.56	0.56	1.0
MPA	10	14	0.13	0.42	3.2
Progesterone	10	17	0.37	0.38	1.0
Chlormadinone acetate	10	19	0.11	0.36	3.3
Hydrocortisone	10	0	0.52	0.56	1.1
None	0	0	0.34	0.34	1.0
TAM	10	24	0.054	0.16	3.0
Verapamil	5	3	0.048	0.30	6.3

a) Indication of additive effect of ADM and each compound.
b) Expected IC_{50} versus actual IC_{50} value.

0.5 μM ADM, TAM (10 μM) potentiated that of 0.063–0.5 μM ADM, and verapamil (5 μM) that of 0.031–0.5 μM ADM.

Effect of MPA on uptake and efflux of ADM The effect of MPA on the uptake of ADM in MCF-7/ADM cells was compared with that of verapamil (Fig. 4A). ADM was accumulated time-dependently in the cells, and its intracellular concentration was 13.3 pmol/10^6 cells after 120 min. This accumulation of ADM was enhanced in the presence of 10 μM MPA or 10 μM verapamil, and after 120 min, the amount of ADM in the cells treated with MPA or verapamil was 1.5 or 2.4 times more than that in the control cells, respectively.

Since the mechanism of ADM resistance of MCF-7/ADM cells was reported to be the expression of P-glycoprotein, which transports ADM from the cells to the extracellular fluid,[22] the influence of MPA on the efflux of ADM was compared with that of verapamil in MCF-7/ADM cells (Fig. 4). ADM, which had accumulated in the cells during the 1-h treatment, was gradually

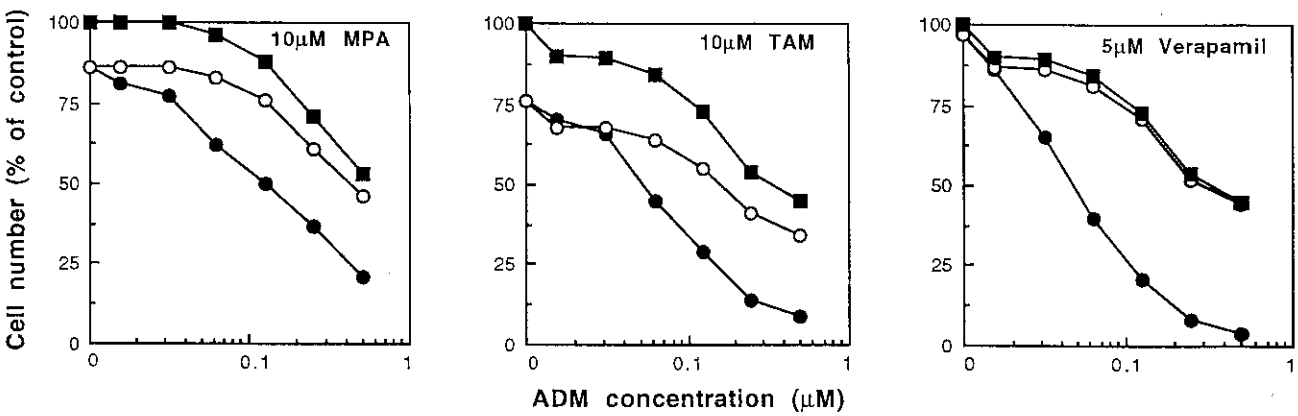

Fig. 3. Effect of MPA, TAM and verapamil on growth-inhibitory activity of ADM against MCF-7/ADM cells. MCF-7/ADM cells (2.5×10^3/well) were cultured on day 0, and treated with ADM alone (■) or in combination with MPA, TAM or verapamil (●) on day 1. The cell number was counted on day 7, and mean values are shown. The standard deviations were less than 10% of mean values. Expected cell number with combination (○).

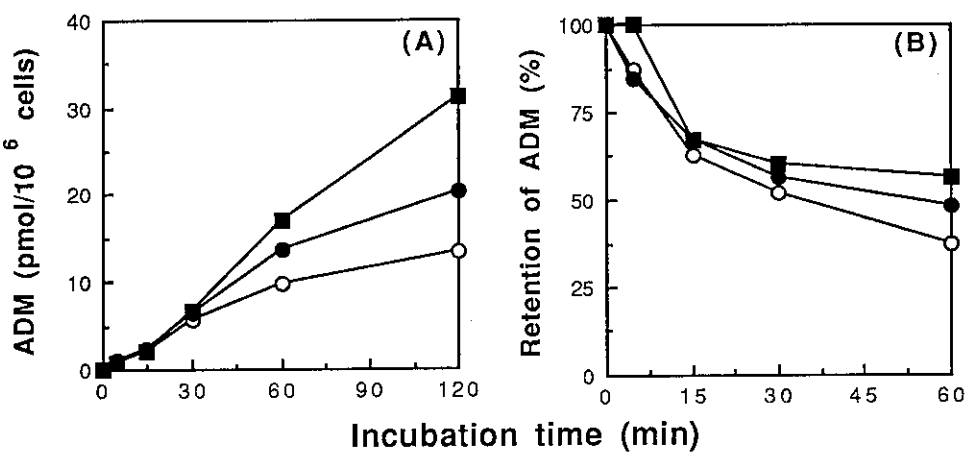

Fig. 4. Effect of MPA and verapamil on uptake and efflux of ADM in MCF-7/ADM cells. (A) The precultured cells (1×10^6/well) were treated with 0.5 μM [^{14}C]ADM alone (○) or in the presence of 10 μM MPA (●) or verapamil (■). At the indicated time, the radioactivity of cells was measured. (B) The precultured cells (1×10^6/well) were treated with 0.5 μM [^{14}C]ADM for 60 min. The cells were then washed and incubated in culture medium alone (○) or in the presence of 10 μM MPA (●) or verapamil (■). At the indicated time, the radioactivity of cells was measured, and mean values are shown. The standard deviations were smaller than the size of the symbols.

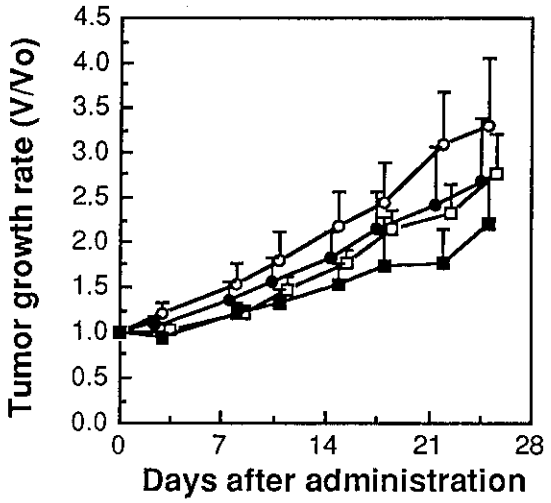

Fig. 5. Antitumor activity of ADM and MPA against MCF-7/ADM cells inoculated into nude mice. When tumors were between 100 and 300 mm^3 (designated as day 0), ADM (13.5 mg/kg) was administered i.v. MPA (126 mg/kg/day) was administered p.o. daily on days 0–4 and 7–11. Each group consisted of 5 mice. ○, untreated; ●, ADM; □, MPA; ■, ADM plus MPA. Mean ± SE is shown.

released from the cells. This efflux of ADM was partially inhibited by treatment with MPA or verapamil, although their effects were not marked, suggesting that MPA as well as verapamil inhibited the function of P-glycoprotein. An experiment on [^{3}H]azidopine photolabeling of P-glycoprotein indicated that MPA binds to P-glycoprotein as well as verapamil (data not shown).

Antitumor activity of ADM and MPA To elucidate whether the *in vitro* combination effect of ADM plus MPA as described above is therapeutically beneficial, an *in vivo* experiment was conducted using nude mice inoculated with MCF-7/ADM cells (Fig. 5). ADM did not significantly inhibit the growth of this tumor, indicating that it retained ADM-resistance even after *in vivo* passage. MPA also did not significantly inhibit the growth of this tumor, presumably due to the lack of high-affinity MPA-binding activity in the tumor. However, when ADM and MPA were used in combination, their antitumor activities were augmented, although statistical significance was not observed versus the ADM- or MPA-treated group because of the large individual variations of tumor size.

DISCUSSION

Concerning the *in vitro* combination effect of ADM and MPA, Shaikh *et al.* demonstrated that the growth-inhibitory activity of ADM against MCF-7 cells was augmented by pretreatment of the cells with MPA.[9] However, the mechanism is unknown. Here we have demonstrated that MPA augmented the growth-inhibitory activity of ADM against ADM-resistant MCF-7 cells (Tables II and III, Figs. 1–3). This effect of MPA was also demonstrated against human nasopharynx carcinoma KB and its ADM-resistant line KB-A1 (Table II). Various steroid hormones were reported to modulate the multidrug resistance by inhibiting the binding of drugs to P-glycoprotein.[20, 21, 27] Among them, progesterone had the highest affinity to P-glycoprotein[20]; interestingly, progesterone is not transported by P-glycoprotein, while other steroids such as cortisol, aldosterone and dexamethasone are.[27] Our results show that MPA modulates ADM resistance more potently than progesterone in MCF-7/ADM cells. This effect of MPA was due to the higher intracellular ADM concentration induced by inhibition of the efflux of ADM from MCF-7/ADM cells (Fig. 4). The efflux of ADM from the cells was observed to some extent even in the presence of MPA or verapamil. It would be interesting to examine whether MPA is transported from the cells by P-glycoprotein.

MCF-7/ADM cells were reported to be negative for expression of estrogen receptors and progesterone receptors.[23] Our results confirmed this, and furthermore, high-affinity MPA-binding activity, which was detected in sensitive MCF-7 cells, was not detected in MCF-7/ADM cells (Table I). The low sensitivity of MCF-7/ADM cells to MPA may be explained by the absence of high-affinity MPA-binding activity. The alternative possibility that the insensitivity of MCF-7/ADM cells to MPA is due to the expression of P-glycoprotein is unlikely if MPA is not transported by P-glycoprotein, as reported previously for progesterone.[27] The high-affinity MPA-binding activity is supposed not to be involved in the mechanism of modulation of multidrug resistance by MPA, since the ADM resistance of MCF-7/ADM cells, which do not express such activity, was modulated by MPA. This speculation is also supported by the results found in KB and KB-A1 cells (Table II). In MCF-7 cells, the synergistic combination effect of ADM and MPA was not observed (Table II). This may be explained by presuming that the IC_{50} value of MPA against MCF-7 cells was too small to modulate the efflux of ADM from MCF-7 cells. The overcoming of ADM resistance of MCF-7/ADM and KB-A1 cells by MPA was incomplete (Table II), suggesting that other mechanisms of ADM resistance may exist in these cell lines.

MCF-7/ADM cells became transplantable in nude mice, and were available for the *in vivo* evaluation of antitumor activity (Fig. 5). ADM and MPA did not inhibit the growth of MCF-7/ADM cells, as predicted from the *in vitro* sensitivity to both drugs. However,

when ADM and MPA were used in combination, their growth-inhibiting activity was augmented, although statistical significance was not observed versus the ADM- or MPA-treated group, suggesting a correlation with the *in vitro* combination effect of ADM and MPA (Table II, Figs. 1–3). The doses of ADM and MPA were 13.5 mg/kg and 126 mg/kg/day, which correspond to about 40 and 380 mg/m^2, respectively, at a conversion rate based on the body surface area for mouse. Actually 40 mg/m^2 of ADM and 600 mg/body of MPA are used clinically for the combination chemotherapy of breast cancers, and they exert sufficient antitumor activity.[6] These results indicate that the clinical advantage of the combination of MPA with ADM against advanced breast cancers may be partly explained by the modulation of ADM resistance by MPA. A comparative study of the pharmacokinetics of ADM and MPA between human and mouse will be helpful as a next step.

ACKNOWLEDGMENTS

We are grateful to Dr. K. H. Cowan and Dr. K. Ueda for providing MCF-7/ADM cells and KB-A1 cells, respectively, and to Ms. Taimi Sano for her excellent technical assistance.

(Received December 2, 1993/Accepted February 16, 1994)

REFERENCES

1) Pannuti, F., Martoni, A., Pollutri, E., Camera, P. and Lenaz, G. R. Medroxyprogesterone acetate (MPA): effects of massive doses in advanced breast cancer. *IRCS Med. Sci.*, **2**, 1605 (1974).
2) Pannuti, F., Martoni, A., Di Marco, A. R., Piana, E., Saccani, F., Becchi, G., Mattioli, G., Barbanti, F., Marra, G. A., Persiani, W., Cacciari, L., Spagnolo, F., Palenzona, D. and Rocchetta, G. Prospective, randomized clinical trial of two different high dosages of medroxyprogesterone acetate (MAP) in the treatment of metastatic breast cancer. *Eur. J. Cancer*, **15**, 593–601 (1979).
3) Anderson, D. G. Management of advanced endometrial adenocarcinoma with medroxyprogesterone acetate. *Am. J. Obstet. Gynecol.*, **92**, 87–99 (1965).
4) Paine, C. H., Wright, F. W. and Ellis, F. The use of progestogen in the treatment of metastatic carcinoma of the kidney and uterine body. *Br. J. Cancer*, **24**, 277–282 (1970).
5) Wils, J. A., Bron, H., Lange, L. V., Pannebakker, M., Romme, A., Scheerder, H., Smeets, J. B. and Beex, L. V. A randomized comparative trial of combined versus alternating therapy with cytostatic drugs and high-dose medroxyprogesterone acetate in advanced breast cancer. *Cancer*, **56**, 1325–1331 (1985).
6) Bruckner, H. W. and Deppe, G. Combination chemotherapy of advanced endometrial adenocarcinoma with adriamycin, cyclophosphamide, 5-fluorouracil, and medroxyprogesterone acetate. *Obstet. Gynecol.*, **50**, 10S–12S (1977).
7) Ishida, H., Okabe, M., Gomi, K. and Horiuchi, R. Effect of medroxyprogesterone acetate on the anticellular activity of 5-fluorouracil against human breast and stomach cancer cells. *Jpn. J. Cancer Chemother.*, **20**, 625–630 (1993) (in Japanese).
8) Ashizawa, T., Ishida, H., Okabe, M. and Gomi, K. Effect of medroxyprogesterone acetate on antitumor efficacies and side effect of 5-fluorouracil. *Jpn. J. Cancer Chemother.*, **20**, 941–947 (1993) (in Japanese).
9) Shaikh, N. A., Owen, A. M., Ghilchik, M. W. and Braunsberg, H. Adriamycin action on human breast cancer cells: enhancement by medroxyprogesterone acetate. *Int. J. Cancer*, **43**, 733–736 (1989).
10) Inaba, M. and Johnson, R. K. Decreased retention of actinomycin D as the basis for cross-resistance in anthracycline-resistant sublines of P388 leukemia. *Cancer Res.*, **37**, 4629–4634 (1977).
11) Skovsgaard, T. Mechanism of cross-resistance between vincristine and daunomycin in Ehrlich ascites tumor cells. *Cancer Res.*, **38**, 4722–4727 (1978).
12) Inaba, M. and Johnson, R. K. Uptake and retention of adriamycin and daunorubicin by sensitive and anthracycline-resistant sublines of P388 leukemia. *Biochem. Pharmacol.*, **27**, 2123–2130 (1978).
13) Fojo, A., Akiyama, S., Gottesman, M. M. and Pastan, I. Reduced drug accumulation in multiply drug-resistant human KB carcinoma cell lines. *Cancer Res.*, **45**, 3002–3007 (1985).
14) Tsuruo, T., Iida-Saito, H., Kawabata, H., Oh-hara, T., Hamada, H. and Utakoji, T. Characteristics of resistance to adriamycin in human myelogenous leukemia K562 resistant to adriamycin and in isolated clones. *Jpn. J. Cancer Res.*, **77**, 682–692 (1986).
15) Shen, D. W., Cardarelli, C., Hwang, J., Cornwell, M., Richert, N., Ishii, S., Pastan, I. and Gottesman, M. M. Multiple drug-resistant human KB carcinoma cells independently selected for high-level resistance to colchicine, adriamycin, or vinblastine show changes in expression of specific proteins. *J. Biol. Chem.*, **261**, 7762–7770 (1986).
16) Tsuruo, T., Iida, H., Tsukagoshi, S. and Sakurai, Y. Overcoming of vincristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res.*, **41**, 1967–1972 (1981).
17) Hofsli, E. and Nissen-Meyer, J. Reversal of multidrug resistance by lipophilic drugs. *Cancer Res.*, **50**, 3997–4002 (1990).
18) Ramu, A., Glaubiger, D. and Fuks, F. Reversal of acquired resistance to doxorubicin in P388 murine leukemia cells by tamoxifen and other triparanol analogues. *Cancer*

Res., **44**, 4392–4395 (1984).
19) Foster, B. J., Grotzinger, K. R., McKoy, W. M., Rubinstein, L., V. and Hamilton, T. C. Modulation of induced resistance to adriamycin in two human breast cancer cell lines with tamoxifen or perhexiline maleate. *Cancer Chemother. Pharmacol.*, **22**, 147–152 (1988).
20) Naito, M., Yusa, K. and Tsuruo, T. Steroid hormones inhibit binding of *Vinca* alkaloid to multidrug resistance related P-glycoprotein. *Biochem. Biophys. Res. Commun.*, **158**, 1066–1071 (1989).
21) Yang, C.-P. H., DePinho, S. G., Greenberger, L. M., Arceci, R. J. and Horwitz, S. B. Progesterone interacts with P-glycoprotein in multidrug-resistant cells and in the endometrium of gravid uterus. *J. Biol. Chem.*, **264**, 782–788 (1989).
22) Fairchild, C. R., Ivy, S. P., Kao-Shan, C.-S., Whang-Peng, J., Rosen, N., Israel, M. A., Melera, P. W., Cowan, K. H. and Goldsmith, M. E. Isolation of amplified and overexpressed DNA sequences from adriamycin-resistant human breast cancer cells. *Cancer Res.*, **47**, 5141–5148 (1987).
23) Vickers, P. J., Dickson, R. B., Shoemaker, R. and Cowan, K. H. A multidrug-resistant MCF-7 human breast cancer cell line which exhibits cross-resistance to antiestrogens and hormone-independent tumor growth *in vivo*. *Mol. Endocrinol.*, **2**, 886–892 (1988).
24) Valeriote, F. and Lin, H. Synergistic interaction of anticancer agents: a cellular perspective. *Cancer Chemother. Rep.*, **59**, 895–900 (1975).
25) Chou, T.-C. and Talalay, P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul.*, **22**, 27–55 (1984).
26) Geran, R. I., Greenberg, N. H., MacDonald, M. M., Schumacher, A. M. and Abbott, B. J. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemother. Rep., Part 3*, **3**, 1–103 (1972).
27) Ueda, K., Okamura, N., Hirai, M., Tanigawara, Y., Saeki, T., Kioka, N., Komano, T. and Hori, R. Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J. Biol. Chem.*, **267**, 24248–24252 (1992).