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MST-16, a novel derivative of bis(2,6-dioxopiperazine), synergistically enhances the antitumor effects of anthracyclines

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Abstract MST-16, a derivative of bis(2,6-dioxopiperazine), is a newly developed anticancer agent that is potentially effective in combination with anthracyclines. It has a structural similarity to ICRF-187. The effects of MST-16 and its active form, ICRF-154, on the cytotoxic activities of six anthracyclines were investigated both in vitro and in vivo. Adriamycin (ADM), therarubicin (THP) and ME2303 (ME) showed synergistic cytotoxicity against colon 26 cells, when combined with MST-16. Epirubicin (EPI) and menogaril (TUT-7) and daunomycin (DM) all had a combination index of less than 1.0 only in the lower fraction affected range and, so there were probably no synergistic interactions between these drugs and MST-16. In colon 26 tumor-bearing mice, a significant delay in tumor growth was noted in the mice treated with ADM (7.5 mg/kg) and MST-16 (750 mg/kg) compared with mice given either drug alone. Similarly, tumor growth in mice treated with THP (10 mg/kg) or ME (10 mg/kg) with MST-16 (750 mg/kg) was significantly delayed. To elucidate the mechanism of synergy between these anthracyclines and MST-16, the concentration of anthracyclines in the treated cells was measured by flow cytometry. No increased intracellular accumulation of ADM, THP or ME was evident even when combined with MST-16. Cell cycle analysis revealed that MST-16 enhanced the accumulation of cells in G_2M induced by ADM, THP and ME 1.6, 1.4, and 1.5 times, respectively. We thus conclude that the administration of ADM, THP and ME combined with MST-16 is synergistic and that the mechanism may not include an increase in the intracellular drug uptake but rather an increase in G_2M accumulation.

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Introduction

MST-16, a derivative of bis(2,6-dioxopiperazine) which can be administered orally, has considerable antitumor activity especially toward adult T-cell leukemia and lymphoma, with response rates reported to be about 43% in clinical trials in Japan [1]. MST-16 is metabolized immediately to its parent compound, ICRF-154, following absorption from the intestine [2]. The basic structure of ICRF-154 is similar to that of ICRF-187 which has a protective effect against cardiotoxicity induced by adriamycin (ADM) without altering the antitumor effect of ADM [3–6]. We have obtained evidence that MST-16 provides almost complete protection against ADM-induced cardio- and renal toxicity in the rat (unpublished data). Thus the therapeutic index of ADM is significantly increased by combined use with MST-16.

Newly developed anthracyclines have been designed in an attempt to reduce ADM-mediated cardiotoxicity, a dose-limiting factor of the drug, while still maintaining its antitumor effect [7, 8]. (2″*R*)-4′-*O*-Tetrahydropyranyladriamycin (THP), a derivative of ADM, is equal to or more effective than ADM [9] against various experimentally induced mouse tumors. This effect of THP is mainly due to a more rapid uptake into tumor cells than seen with ADM [10]. The cytotoxic effect of THP on hamster cardiac tissue is also weaker than that of ADM using the same dose [11]. ME2303 [ME, 7-*O*-(2,6-dideoxy-2-fluoro-α-L-talopyranosyl)-adriamycinone-14-*O*-pimelate] has a superior cytotoxic effect to ADM against various murine tumor models over a wide range of dosages, and is also effective against multidrug-resistant human tumor cell lines and their parental cell lines in vitro [12]. 4′-Epiadriamycin (EPI) and daunomycin (DM) are now widely used to treat patients with cancer [13]. Menogaril (TUT-7, TUT), a novel anthracycline derivative, is now in clinical trials in the United States

[14, 15]. The effect of MST-16 on the cytotoxic actions of these anthracyclines has only been examined for ADM.

We investigated the interaction of MST-16 and these six anthracyclines (ADM, EPI, THP, TUT, ME and DM) both in vitro and in vivo, with the objective of determining whether or not MST-16 could increase the antitumor effect of these drugs.

Materials and methods

Drugs

MST-16 and ICRF-154, kindly provided by the Zenyaku Kogyo Co. (Tokyo, Japan), were dissolved in dimethyl sulfoxide (DMSO) for the cytotoxic assays. MST-16 was suspended in a 0.5% hydroxypropylmethylcellulose (HPMC, Shin-Etsu Chemical Co., Tokyo, Japan) solution for oral administration to mice. ADM and EPI were obtained from the Kyowa Hakko Kogyo Co. (Tokyo, Japan), THP, ME and DM from the Meiji Seika Co. (Tokyo, Japan), and TUT from the Taiho Yakuhin Kogyo Co. (Tokyo, Japan). These six anthracyclines were dissolved in normal saline just before use in the cytotoxic assays.

Animals

Female BALB/c mice, at 5 weeks of age, were purchased from Charles River Japan (Kanagawa, Japan), housed in plastic cages, and fed a laboratory diet and water ad libitum. Before starting the experiment, the mice were left for at least 1 week to adapt to the environment.

Cell lines

Colon 26 cells, a murine colon adenocarcinoma, were kindly provided by the Zenyaku Kogyo Co., and were maintained in plastic culture flasks containing an RPMI-1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with penicillin (200 units/ml), gentamicin (0.04 mg/ml), streptomycin (0.1 mg/ml), and 10% heat-inactivated fetal calf serum (FCS, Bioserum Co., Victoria, Australia).

Cytotoxicity assay in vitro

The cytotoxic activity of the drugs was measured using an MTT assay. Exponentially growing cells were harvested, counted using trypan blue exclusion, and then resuspended to an appropriate concentration. The cell suspensions (10^4/100 μl) were dispensed into a replicate 96-well culture plate with a lid (Corning 25860, N.Y.). Next, 0.1 ml culture medium containing the drugs at the appropriate concentrations, or culture medium containing the drug vehicle, was dispensed into the appropriate wells (vehicle control group, $n = 11$; each drug treatment group, $n = 4$) using a multichannel pipette. After 3 days of incubation, 0.01 ml MTT solution (0.4% in phosphate-buffered saline, PBS) and the same volume of a sodium succinate solution (0.1 *M* in PBS) were dispensed into each well following the removal of the culture medium. The culture plate was incubated for 3 h prior to the addition of DMSO (0.15 ml). The absorbency was measured using a microtiter culture plate reader (Easy Reader model EAR 340, SY-LAB, Purkersdorf, Austria) soon after shaking the plate for 5 min with a plate mixer (Micro Mixer model MX-4, Sanko Junyaku Co., Tokyo, Japan).

Analysis of cytotoxic interaction between two drugs

The cytotoxic interaction between two drugs was evaluated using the median effect analysis developed by Chou and Talalay [16, 17]. The fraction affected with the combined drugs (Fa) was defined as:

$$\mathrm{Fa} = 1 - \frac{(\text{OD of treatment cells})}{(\text{OD of control cells})}$$

A graph was made of the median-effect plot and the median dose concentration (Dm), i.e. ED_{50}, LD_{50} and IC_{50}, of the respective drugs and their mixtures were determined, m is a coefficient signifying the sigmoidicity of the dose-effect curve. In this experiment, if the slopes (*m*) of the two drugs obtained by the plots were not parallel to each other, the interaction of the two drugs was taken as nonexclusive. The combination index (CI) was obtained using the formula:

$$\mathrm{CI} = \frac{(\mathrm{D})_1}{(\mathrm{Dx})_1} + \frac{(\mathrm{D})_2}{(\mathrm{Dx})_2} + \frac{(\mathrm{D})_1(\mathrm{D})_2}{(\mathrm{Dx})_1(\mathrm{Dx})_2}$$

where $(D)_1$ and $(D)_2$ represent the contributions of drugs 1 and 2 in the mixture $(Dx)_{1,2}$ from the known dose ratios of the two drugs, and (Dx) is defined as follows:

$$\mathrm{Dx} = \mathrm{Dm}[\mathrm{Fa}/(1 - \mathrm{Fa})]^{1/\mathrm{m}}$$

Values of the CI < 1.0 were taken as indicating synergism, CI = 1.0 as additivity and CI > 1.0 as antagonism.

Antitumor effect and normal tissue toxicity in vivo

The effect on tumor growth of MST-16 either alone or in combination with anthracyclines was investigated using mice bearing subcutaneous colon 26 adenocarcinoma. A suspension of viable tumor cells (10^6 cells in 0.2 ml Hank's balanced salt solution HBSS) was prepared from the exponentially growing tumor cells and was inoculated into the right flank of each mouse; the tumor grew in all animals. The drugs were administered when tumors reached an estimated volume of 100–150 mm^3. As shown in Table 1, 75 tumor bearing mice were divided into groups of either 8 mice (groups 1, 2 and 4) or 9 mice (groups 3, 5, 6, 7 and 8). Each mouse was treated orally with either HPMC (0.1 ml/10 g) or MST-16 (750 mg/kg) followed 60 min. later by an intraperitoneal injection of either saline (0.1 ml/10 g), ADM (7.5 mg/kg), THP (10 mg/kg) or ME (10 mg/kg).

The tumor volume (mm^3, TV) was calculated from the formula:

$$\mathrm{TV} = \frac{\mathrm{AB}^2}{2}$$

where A and B represent the maximal and minimal diameter (mm) of the tumor, respectively. The tumors were measured daily using a slide caliper. A relative TV was thus calculated from the formula:

$$\text{Relative TV} = \frac{\mathrm{TV}}{\mathrm{TV}_0}$$

where TV_0 is the TV at the time of treatment. The tumor growth time (TGT) was defined as the time taken for the relative TV to reach 3.0. The tumor growth delay (TGD), defined as the difference in TGT between the treatment group and the control group, was used to evaluate response of the tumor to treatment.

As an indicator of the side effects of treatment, the body weight (BW) of the mice was measured daily and the relative body weight was calculated using the formula:

$$\text{Relative BW} = \frac{\mathrm{BW}}{\mathrm{BW}_0} \times 100(\%)$$

where BW^0 is the initial body weight at the time of treatment.

Flow cytometry

Intracellular concentrations of anthracyclines

Colon 26 cells in early exponential growth were incubated with either 100 μ*M* MST-16 or 1 μ*M* anthracycline (ADM, THP or

Table 1 Combined effect of MST-16 and anthracyclines against colon 26 tumors in mice

Treatment group	Tumor growth time[a] (days)	Tumor growth delay (days)		Relative body weight[d] (%)
		Actual[b]	Simple additive[c]	
Control	4.3 ± 0.6	0		99.4
MST-16 (750 mg/kg)	5.8 ± 0.8	1.52 ± 0.92		101.3
ADM (7.5 mg/kg)	7.1 ± 1.1	2.75 ± 1.09		99.4
ADM + MST-16	10.0 ± 1.7*	5.71 ± 0.45*	4.27	99.6
THP (1 mg/kg)	6.1 ± 0.6	1.86 ± 0.63		103.9
THP + MST-16	11.4 ± 1.7*	7.13 ± 1.73*	3.38	103.4
ME (10 mg/kg)	5.9 ± 1.0	2.02 ± 1.66		108.2
ME + MST-16	11.4 ± 1.5*	7.14 ± 1.85*	3.54	105.3

* $P < 0.05$
[a] Time required to reach three times the initial tumor volume
[b] $TGT_{\text{treatment group}} - TGT_{\text{control group}}$
[c] $TGD_{\text{MST group}} + TGD_{\text{anthracycline group}}$
[d] $BW_{\text{day8}}/BW_{\text{day0}} \times 100$ (%)

ME) alone or in combination for 0.5, 1, 1.5, 2, 6, 12 and 24 h in RPMI-1640 medium with 10% FCS. The cells were washed twice and then resuspended in PBS at a final concentration of 1×10^5 cells/ml. The cell size and fluorescence intensity were determined using a flow cytometer (FACScan/CellFIT, Becton Dickinson, San Jose, Calif.). The cells (1×10^4) were analyzed with Consort 30 software at a flow rate of 50–150 cells/s. In each experiment, the fluorescence intensity was measured with excitation at 488 nm (1 W power) and emission integrated above 530 nm. The cells were selected by gating the scattered light from the cell suspension. The mean cellular fluorescence was determined by calculating the fluorescence distribution and subtracting the spontaneous fluorescence.

Cell cycle study

Colon 26 cells in early exponential growth were incubated with either 10 μ*M* MST-16 or 0.1 μ*M* anthracycline (ADM, THP or ME) or both for 24 h in RPMI-1640 medium with 10% FCS. The cells were washed twice in PBS and suspended in 0.2% (v/v) Triton-X-100 detergent (Katayama Chemical Co., Osaka, Japan), and then left at room temperature for 20 min. After vigorous pipetting to rupture the cell membranes and to remove the cytoplasm and RNA, 100 μg/ml propidium iodide (PI, Sigma Chemical Co., St. Louis, Mo.) in sodium citrate buffer and 100 μl 0.5 mg/ml ribonuclease A (RNAse, Sigma) were added to the cell suspension at a concentration of 10^6 cells/ml and the preparation was then incubated in the dark for 30 min. A flow cytometer was used to count 10 000 nuclei. CellFIT software was used for data analysis at excitation and emission wavelengths of 488 and 590 nm, respectively. A histogram of the red fluorescence of DNA was made with coefficients of variation of less than 5%.

Statistics

The difference in TGT and relative BW among the eight groups of mice was evaluated using Student's *t*-test. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

Cytotoxic effects of combinations with anthracyclines and MST-16 in vitro

With the molar ratios between the anthracyclines and MST-16 or ICRF-154 varying between 1:10 and 1:200, the maximal synergistic effect was attained with a ratio of 1:100. Among the six anthracyclines, ADM, THP and ME showed CIs of less than 1.0, with an Fa range from 0.4 to 0.9 when combined with MST-16 (Fig. 1). The cytotoxic interactions between these three anthracyclines and MST-16 were thus considered to be synergistic.

However, for the three other anthracyclines, EPI, DM and TUT, the combination indices were less than 1.0, with low Fa values. Thus no synergistic interactions occurred between these three drugs and MST-16.

In the case of ICRF-154, the combination with ADM, EPI or THP was considered to be synergistic, and with TUT or DM antagonistic, with a range of Fa from 0.5 to 0.9 (Table 2). The combination of ICRF-154 and ME thus had an additive effect.

Tumor growth delay assay

Three anthracyclines (ADM, THP and ME) acted synergistically with MST-16 in vitro, and were used for in vivo studies. Figure 2 shows the tumor growth curves following treatment with MST-16 and these three anthracyclines. Combined treatment with MST-16 and the three anthracyclines (groups 4, 6 and 8) was more effective against the growth of the tumor than vehicle-only treatment (group 1) and any single-drug treatment (groups 2, 3, 5 and 7). As shown in Table 1, MST-16 or ADM alone produced minimal antitumor effects with a TGD of 1.52 and 2.75 days, respectively. However, when administered in combination (ADM plus MST-16, group 4) produced a significant antitumor effect with a TGD of 5.71 days.

Similar results were obtained in the groups of mice given either THP or ME alone, which produced a TGD of 1.86 or 2.02 days, respectively. In combination with MST-16, the antitumor effects of THP (group 6) and ME (group 8) were significantly greater than those of either drug alone, with a TGD of 7.13 and 7.14 days, respectively.

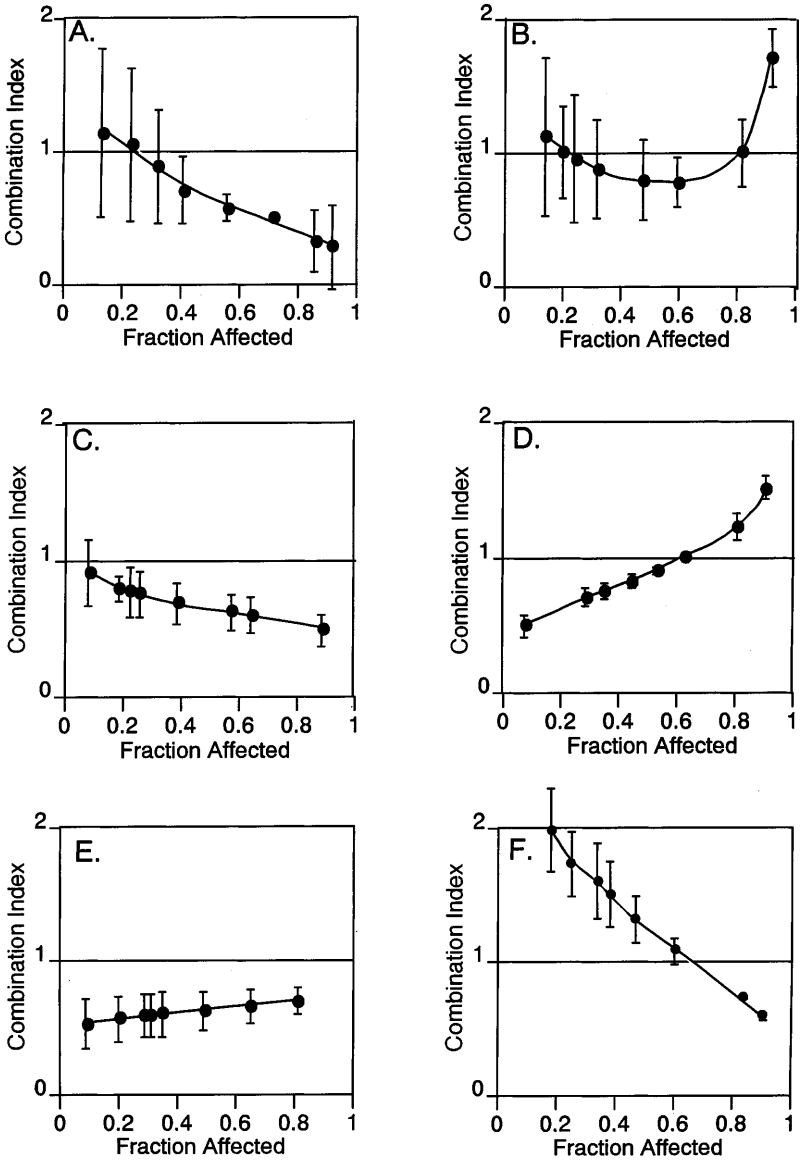

Fig. 1A–F Combination indices indicating the cytotoxic interactions between anthracyclines and MST-16 against colon 26 cells at each level of cytotoxicity (fraction affected, Fa) shown on the horizontal axis. When the value is less than 1, the interaction of two drugs is judged to be synergistic. When the value is equal to or more than 1, the interaction is considered to be additive and antagonistic, respectively (**A** ADM and MST-16, **B** EPI and MST-16, **C** THP and MST-16, **D** TUT and MST-16, **E** ME and MST-16, **F** DM and MST-16; *bars* standard deviation)

To evaluate the enhancement of the antitumor effect of these anthracyclines by MST-16, we used the following formula;

$$A = TGD_{MST-16\ alone} + TGD_{ADM,THP\ or\ ME\ alone}$$

where A represents the estimated additive antitumor effect of MST-16 and ADM, THP or ME. As shown in Table 1, the actual values of TGD for the combined treatments with MST-16 and anthracyclines (B) were compared with A. The increases in TGD thus determined were 1.44 for ADM, 3.75 for ME and 3.60 for THP. These three agents thus had a more than additive antitumor effect a when given in combination with MST-16.

Table 2 Combination indices of anthracyclines with ICRF-154 (*Fa* fraction affected)

Anthracycline	Fa 50%	Fa 70%	Fa 90%
ADM	0.77 ± 0.21	0.66 ± 0.12	0.58 ± 0.07
EPI	0.97 ± 0.21	0.82 ± 0.08	0.72 ± 0.16
THP	0.52 ± 0.11	0.61 ± 0.08	0.84 ± 0.17
TUT	0.20 ± 0.11	0.71 ± 0.18	10.5 ± 5.34
ME	0.47 ± 0.16	0.63 ± 0.16	1.01 ± 0.31
DM	4.55 ± 1.92	3.28 ± 0.97	2.26 ± 0.47

Body weight change

The relative BWs 8 days after each treatment are shown in Table 1. In the treated and control groups, the findings were much the same.

Effect on the survival period of the treated mice

The mean survival periods of the tumor-bearing mice treated with the three anthracyclines alone were 32.6,

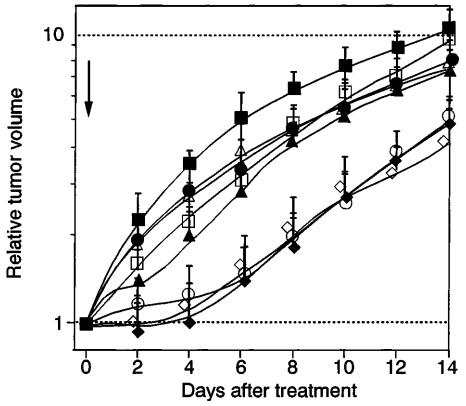

Fig. 2 Tumor growth curves after treatment as demonstrated by the relative tumor volume calculated in relation to the initial volume. (■ control, ● MST-16 750 mg/kg, ▲ ADM 7.5 mg/kg, ♦ ADM 7.5 mg/kg + MST-16 750 mg/kg, □ THP 10 mg/kg, ○ THP 10 mg/kg + MST-16 750 mg/kg, △ ME 10 mg/kg, ◇ ME 10 mg/kg + MST-16 750 mg/kg; *bars* standard deviation)

34.0 and 37.4 days, respectively, while for the control mice it was 29.0 days. The addition of MST-16 to ADM, THP or ME significantly prolonged survival to 42.3, 36.1 and 42.7 days, respectively.

Intracellular accumulation of the drugs

To examine the mechanism of the enhanced antitumor effect of ADM, THP and ME by MST-16 the intracellular concentrations and cell cycle traverse were measured by flow cytometry. When the colon 26 cells were incubated with anthracyclines, either with or without MST-16, no differences were seen in the intracellular accumulation of ADM (Fig. 3). For THP and ME, little difference was observed in the antitumor effects with or without MST-16.

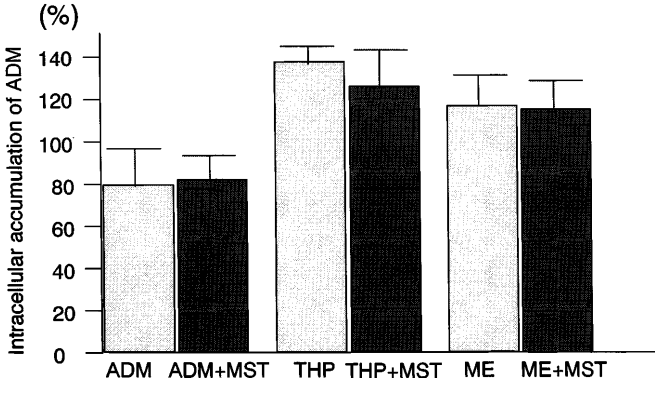

Fig. 3 Intracellular concentration of anthracyclines with or without MST-16. Colon 26 cells were exposed to anthracyclines (1 μ*M*) with or without MST-16 (100 μ*M*) for 1 h. The intracellular concentration of anthracyclines was measured by flow cytometry (*bars* standard deviation)

Table 3 Combined effects of MST-16 and anthracyclines on the cell kinetics of colon 26 cells. Values are percentages of each cell cycle fraction

	G_0/G_1	S	G_2M
Control	61.2 ± 1.3	23.7 ± 2.2	15.1 ± 1.6
MST-16 (10 μ*M*)	51.7 ± 2.4	28.2 ± 3.1	20.2 ± 1.7*
ADM (0.1 μ*M*)	44.9 ± 6.9	37.0 ± 2.6	18.1 ± 3.1
ADM + MST-16	37.9 ± 1.2	34.2 ± 1.8	27.9 ± 2.4*
THP (0.1 μ*M*)	40.7 ± 4.0	25.9 ± 3.7	33.5 ± 2.3
THP + MST-16	39.3 ± 5.7	14.7 ± 1.3	45.0 ± 2.4*
ME (0.1 μ*M*)	46.5 ± 4.0	27.8 ± 2.7	25.7 ± 3.3
ME + MST-16	37.8 ± 4.9	25.1 ± 3.9	37.2 ± 2.6*

* $P < 0.05$

Cell cycle analysis

Cell cycle analysis revealed that MST-16 (10 μ*M*) increased the G_2M accumulation induced by ADM, THP and ME (0.1 μ*M*, continuously for 24 h) by 18.1%, 33.5% and 25.7% to 27.9%, 35.0% and 37.2%, respectively, as shown in Table 3.

Discussion

Anthracyclines have a broad spectrum of antitumor activity against human cancer. However, cardiotoxicity, the dose-limiting factor of anthracyclines, which is characterized by diffuse, irreversible myocardial injury, limits the clinical usefulness of these compounds [18]. Preclinical studies suggest that there are separate mechanisms for the antitumor activity and cardio toxicity of anthracyclines, and this has led to the development of such potentially cardioprotective agents as ICRF-187, which interferes with anthracycline-induced iron-mediated free radical generation [3, 4]. We have previously obtained evidence that MST-16 also provides almost complete protection against ADM-induced cardio- and renal toxicity in the rat (unpublished data). MST-16 thus seems to be a potentially useful agent for increasing the therapeutic index of ADM.

Some bis-dioxopiperazines enhance the antitumor activity of ADM [5]. However, the cytotoxic interactions of MST-16 and newly developed anthracyclines have yet to be clarified. In the present study, MST-16 increased the cytotoxicity of the three anthracyclines studied both in vitro and in vivo, and also prolonged the survival of tumor bearing mice. On the other hand, the body weight did not differ from the vehicle-treated controls even after the combined treatments. The therapeutic indices of these three anthracyclines were thus greatly increased by MST-16.

Both THP and ME are new anthracycline derivatives which have demonstrated excellent antitumor activity in various experimental and clinical studies [12, 19, 20]. THP has been found to be more effective against advanced breast cancers and has less cardiotoxicity. ME is

effective against multidrug-resistant cell lines, because of structural replacement of the C'-3 amino group by fluorine. One reason for the differences in cytotoxicity between ADM, THP and ME combined with MST-16 may be the potential to produce free radicals. THP and ME were mainly synthesized in an attempt to decrease the toxicity of ADM against normal tissue, especially cardiotoxicity, mainly caused by ADM-mediated free radicals [7, 10–12]. The combination of these two drugs with MST-16 would be less effective than combinations with ADM and MST-16 if the free radicals play a role in the antitumor effects in vitro and in vivo. We noted that the antitumor activities of THP and ME were greater than that of ADM in combination with MST-16, DNA intercalation and the inhibiting action of topoisomerase II. In addition, differences in drug delivery to tumor tissue or intracellular accumulation may also explain these differences.

The mechanism of enhancement of the antitumor effect of anthracyclines by MST-16 is still not clear. We examined whether MST-16 might change the intracellular uptake of anthracyclines and enhance the cytotoxicity of the agents. Using flow cytometry in vitro, we found that MST-16 did not affect the intracellular accumulation of any of the anthracyclines studied. This indicates that MST-16 may not affect the drug delivery of anthracyclines to cells, nor the drug uptake or release by cells, but it did influence anthracycline induced cytotoxic actions including DNA intercalation and inhibition of topoisomerase II activity. Both MST-16 and anthracyclines are topoisomerase II inhibitors, but MST-16, a masked compound of ICRF-154, inhibits topoisomerase II activity in a different manner from anthracyclines [21]. MST-16 limits the activity of topoisomerase II to inhibit the production of a cleavable DNA–protein complex, and thus differs from other topoisomerase II inhibitors such as anthracyclines [22]. This different action may be one reason for the enhanced effect of MST-16 combined with these three anthracyclines.

Regarding the mechanisms of the synergistic cytotoxic action of combinations of MST-16 and anthracyclines, we demonstrated that the proportion of cells in the G_2M phase increased significantly after combined administration in comparison with the drugs alone. Although tumor cells are arrested in the G_2M phase by anthracyclines [23] and by MST-16 [24], the drug-sensitive phase differs between the two types of drug [21, 22, 25, 26]. Anthracyclines produce a gradual increase in the inhibition of DNA synthesis from the G_1 to the G_2M phase, thus demonstrating the cell cycle phase dependency of the drug toxicity [26], while ICRF-154 affects cells in the G_2M phase [22]. Wadler et al. [27] reported that the combination of ICRF-187 and doxorubicin enhance cell cycle blockade in the G_2M phase. This combined effect of ADM and ICRF-187 may be related to the potential of ICRF-187 to increase the proportion of cells in G_2, making the cells relatively more accessible to the effects of anthracycline, and to deplete the G_1 population, which is relatively anthracycline-resistant. Similarly, the cells affected by MST-16 also accumulate in the late G2 phase, which may also explain the enhanced cytotoxicity of MST-16 combined with anthracyclines both in vitro and in vivo.

In this study, we demonstrated a significantly prolonged survival of tumor-bearing mice treated with anthracyclines combined with MST-16, compared with mice treated with the drugs alone, and that MST-16 synergistically enhanced the cytotoxicity of anthracyclines both in vitro and in vivo. The prolonged survival as well as the enhanced antitumor activity induced by the combination treatment could be clinically useful and deserves further examination in a clinical setting.

In conclusion, MST-16 was found to synergistically enhance the cytotoxic effects of ADM, THP and ME both in vitro and in vivo and a possible mechanism of this action is an interaction of anthracyclines and MST-16 that leads to accumulation of tumor cells in the G_2M phase.

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