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Comparison of antitumor activity of new anthracycline analogues, ME2303, KRN8602, and SM5887 using human lung cancer cell lines.

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Abstract

In an attempt to predict the clinical activity of newly developed anthracycline analogues, ME2303, KRN8602, and SM5887 in the treatment of lung cancer, we compared antitumor activity of these drugs with that of adriamycin, using six human lung cancer cell lines and two drug-resistant human lung cancer sublines. Taking the pharmacokinetic data into consideration, we evaluated the relative antitumor activity: the ratio of area under the concentration-time curve of each drug to the 50% inhibitory concentration of the drug. Regarding this ratio, ME2303 was more potent than adriamycin, SM5887, and KRN8602. Cross-resistance of the new analogues to adriamycin was investigated using an adriamycin-resistant small cell lung cancer subline, SBC-3/ADM100 and an etoposide-resistant subline, SBC-3/ETP. SBC-3/ADM100 being 106-fold more resistant to adriamycin than the parent SBC-3 showed less resistance to the analogues: 1.80-fold to KRN8602, 3.80-fold to SM5887, and 8.60-fold to ME2303. SBC-3/ETP which was 52.1-fold more resistant to etoposide and 39.5-fold more resistant to adriamycin were also less resistant to the new analogues: 3.27-fold to KRN8602, 9.07-fold to SM5887, and 17.3-fold to ME2303. In conclusion, ME2303 was found to be the most potent agent among drugs tested for the treatment of lung cancer, and KRN8602 can be expected to be beneficial for the treatment of drug-resistant small cell lung cancer.

KEYWORDS: new anthracycline analogues, ME2303, KRN8602, SM5887, lung cancer cell line

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In an attempt to predict the clinical activity of newly developed anthracycline analogues, ME2303, KRN8602, and SM5887 in the treatment of lung cancer, we compared antitumor activity of these drugs with that of adriamycin, using six human lung cancer cell lines and two drug-resistant human lung cancer sublines. Taking the pharmacokinetic data into consideration, we evaluated the relative antitumor activity: the ratio of area under the concentration-time curve of each drug to the 50% inhibitory concentration of the drug. Regarding this ratio, ME2303 was more potent than adriamycin, SM5887, and KRN8602. Cross-resistance of the new analogues to adriamycin was investigated using an adriamycin-resistant small cell lung cancer subline, SBC-3/ADM100 and an etoposide-resistant subline, SBC-3/ETP. SBC-3/ADM100 being 106-fold more resistant to adriamycin than the parent SBC-3 showed less resistance to the analogues: 1.80-fold to KRN8602, 3.80-fold to SM5887, and 8.60-fold to ME2303. SBC-3/ETP which was 52.1-fold more resistant to etoposide and 39.5-fold more resistant to adriamycin were also less resistant to the new analogues: 3.27-fold to KRN8602, 9.07-fold to SM5887, and 17.3-fold to ME2303. In conclusion, ME2303 was found to be the most potent agent among drugs tested for the treatment of lung cancer, and KRN8602 can be expected to be beneficial for the treatment of drug-resistant small cell lung cancer.

Key words : **new anthracycline analogues, ME2303, KRN8602, SM5887, lung cancer cell line**

During the last decade, the beneficial role of chemotherapy in the treatment of small cell lung cancer (SCLC) has been confirmed. More than 90% of patients receiving current chemotherapy have achieved significant clinical response. However, the majority of responding patients eventually relapse and less than 20% of those will survive more than two years (1). Regarding non-small cell lung cancer (NSCLC), chemotherapy is only marginally effective and the role of chemotherapy is still controversial (2). In this situation, it becomes progressingly important to develop more potent and less toxic agents for the treatment of lung cancer.

Adriamycin (ADM), an anthracycline antibiotic, is a highly active agent for a wide variety of malignancies including SCLC (3), however, durable responses were extremely rare in case of SCLC. Development of multidrug resistance (MDR) is considered to be a major obstacle to the

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achievement of satisfactory outcomes in SCLC (4). In addition, cumulative cardiotoxicity has limited the broad use of the drug (5). Therefore, a major effort has been focused on synthesizing or isolating an analogue which is more active and less cardiotoxic, and exerts significant action against cells overexpressing the MDR1 gene (6). Recently, several new anthracycline analogues showing greater activity than ADM in experimental tumors have been developed in Japan. These include ME2303, 2-fluoroglycosidated ADM (7, 8), KRN8602, a morpholino anthracycline (9), and SM5887, being characterized by the 9-amino group and a simple sugar moiety different from daunosamine (10). In this study, we attempted to compare the activity of the newly developed anthracycline analogues using human lung cancer cell lines in order to predict their clinical efficacy in the treatment of lung cancer.

Materials and Methods

Chemical agents. The drugs used in the experiments were provided by the following sources: ADM from Kyowa Hakko Co., Ltd., Tokyo, Japan; ME2303 and its metabolite, ME2303-M-1 from Meiji Seika Co., Ltd., Tokyo, Japan; KRN8602 (MX-2) and its metabolite KRN8602-M2, from Kirin Co., Ltd., Tokyo, Japan; SM5887 and its metabolite, SM5887-13-OH, from Sumitomo Co., Ltd., Osaka, Japan. 3-[4, 5-dimethyl-thiazol-2-yl] 2, 5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Chemical Co., St. Louis, MO, USA.

Fig. 1 illustrates chemical structures of the new analogues, ME2303: (8S, 10S)-8-(6-carboxyhexanoyloxyacetyl)-10-[(2, 6-dideoxy-2-fluoro-α-L-talopyranosyl) oxy] -7, 8, 9, 10-tetrahydro-6, 8, 11-trihydroxy-1-methoxy-5, 12-naphthacenedione, KRN8602: 3′-deamino-3′-morpholino-13-deoxo-10-hydroxycarminomycin hydrochloride, SM5887: (7S, 9S)-9-acetyl-9-amino-7-[(2-deoxy-β-D-erythro-pentopyranosyl) oxy] -7, 8, 9,

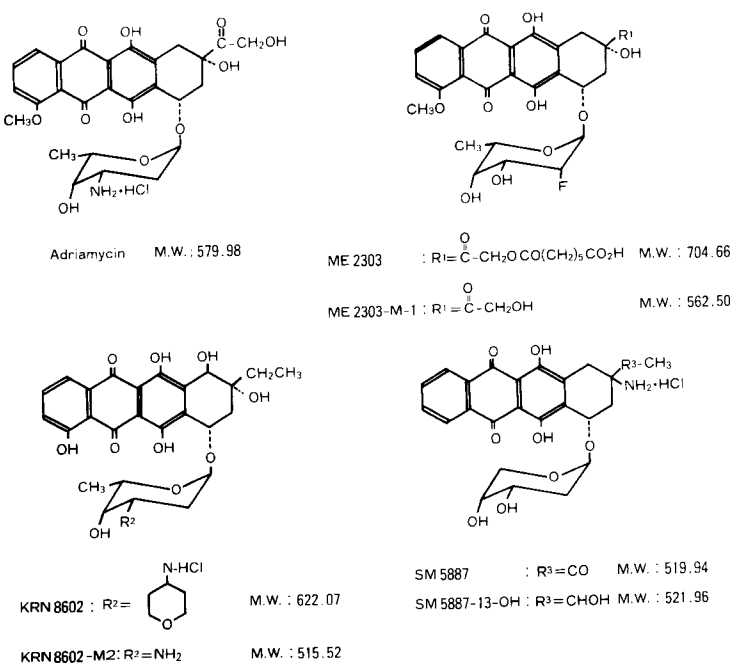

Fig. 1 Chemical structures of adriamycin, ME2303, KRN8602, SM5887, and their metabolites.

10-tetrahydro-6, 11-dihydroxy-5, 12-naphthacenedione hydrochloride.

ADM and SM5887 were dissolved in 0.9% saline. ME2303 was dissolved in phosphate buffer saline (PBS). ME2303-M-1 was dissolved in dimethylsulfoxide. KRN8602 and SM5887-13-OH were dissolved in distilled water. KRN8602-M2 was dissolved in equimolar HCl. ME2303-M-1 solution was stored at 4°C, and the other drug solutions were stored at −20°C. These drug solutions were diluted with RPMI1640 (GIBCO, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) immediately before use.

Cell cultures. Human lung cancer cell lines used in the experiments were SBC-2, -3, -4, -7, ABC-1, EBC-1, all of which were established in our laboratory (Table 1) (11, 12). SBC-3/ADM100 (13), a highly resistant cell line to ADM, was derived from SBC-3/ADM (14) *in vitro* by continuous exposure to increasing concentrations of ADM with cloning procedures in soft agar. SBC-3/ETP, markedly resistant to etoposide (ETP), was derived from the parent SBC-3 using the same procedures (15). The SBC-3/ADM100 and the SBC-3/ETP overexpressed P-glycoprotein, while the parent SBC-3 did not display the substance at all by flow-cytometric analysis using MRK16 (13, 15), an antibody to P-glycoprotein, which was kindly provided by Dr. T. Tsuruo in the Applied Microbial Institute, Tokyo University, Japan. Intracellular GST-π contents of SBC-3/ADM100 and SBC-3/ETP were elevated compared to that of the SBC-3. Both topoisomerase I and II activities in nuclear extracts from SBC-3/ADM100 and SBC-3/ETP were lower than those in SBC-3 (13, 15).

All the cell lines were maintained in tissue culture flasks (Costar 3055) at 37°C in a humidified atmosphere supplemented with 5% CO_2. The medium used was RPMI-1640 supplemented with 10% FBS, penicillin-G (100 units/ml), and streptomycin (100 μg/ml), (RPMI-FBS).

Assay of drug sensitivity. Drug sensitivity was measured by the MTT assay, which was modified from the original method described by Mosmann (16). Fifty microliters RPMI-FBS containing serial concentrations of chemotherapeutic agents and 50 μl RPMI-FBS without agents were prepared in 96-well flat-bottomed microplates (Costar). Fifty microliters of tumor cell suspension containing approximately 5,000 cells in RPMI-FBS was plated to each well of the microplates, and incubated for 96 h at 37°C in a humidified atmosphere with 5% CO_2. Then, 10 μl PBS containing 50 μg MTT was added to each well, and incubated for 4 h. After addition of 125 μl fresh isopropanol with 0.04 N HCl to each well, the microplates were vigorously shaken using the Direct Mix Model TS-50 (Thermal Kagaku Sangyo Co., Ltd., Tokyo) for 2 min. The absorbance of the wells at 560 nm was measured using the Model 3550 microplate reader (Bio-Rad Laboratories, CA, USA). The absorbance of wells containing drug-free medium without tumor cells was measured as background, and the absorbance of wells containing drug-free medium with tumor cells was measured as a control. The experiment was repeated at least 3 times for each drug. The surviving cell fraction was calculated by the following formula: [(mean absorbance in four test wells − absorbance in background wells)/(mean absorbance in control wells − absorbance in background wells)] × 100.

The concentration of each drug necessary to inhibit the growth of tumor cells by 50% (IC50) was determined by plotting the surviving fraction to a drug concentration on a semi-log section paper. Relative antitumor activity (RAA) of each drug was calculated by the following formula: [RAA] = Area under the concentration-time curve [AUC] (μM × h) at a recommended dose in

Table 1 Characteristics of lung cancer cell lines.

Lung cancer cell line	Histological type	Previous treatment status	Source
SBC-2	Small, classic	COMP, VAN, IFX, VDS	Bone marrow
SBC-3	Small, variant	Untreated	Bone marrow
SBC-4	Small, variant	COMP, VAN, ACR	Bone marrow
SBC-7	Small, variant	COMP, VAN, IFX, CDDP, VDS	Pericardial effusion
ABC-1	Adenocarcinoma	COMP, MMC	Pleural effusion
EBC-1	Squamous	Untreated	Skin metastasis

COMP: Combination of cyclophosphamide, vincristine, methotrexate, and procarbazin, VAN: Combination of VP-16, adriamycin, and nimustine, IFX: ifosfamide, VDS: vindesine, ACR: aclarubicin, CDDP: cisplatin, MMC: mitomycin C

Table 2 Clinically recommended dose and AUC of adriamycin and new anthracycline analogues.

	Recommended dose (mg/m²)	AUC (μM•hour)
Adriamycin	50	2.46
ME2303	160	4.87
ME2303-M-1		5.71
KRN8602	30	0.765
KRN8602-M2		0.0514
SM5887	100	12.6
SM5887-13-OH		1.32

man/IC50 value (nM) for the corresponding drug determined by MTT assay. The AUCs of ME2303, ME2303-M-1, KRN8602, KRN8602-M2, SM5887, and SM5887-13-OH were reported in previous phase I studies (17, 18, 19), and the AUC of ADM was derived from the report of Erttmann *et al.* (20) (Table 2).

Results

Comparison of the antitumor activity of ADM, new anthracycline analogues and their metabolites. All the drugs showed a cytotoxic effect in a dose-dependent manner within the concentration ranges tested in the individual experiments. In order to evaluate the antitumor activity of the drugs, we compared the RAA of each drug by determining the ratio of the AUC to the IC50 of the drug measured by MTT assay. The IC50 values and the RAA of all the drugs tested using four SCLC cell lines and two NSCLC cell lines are listed in Table 3. In general, SBC-3 was the most susceptible cell line to chemotherapeutic agents, followed by ABC-1, SBC-2, EBC-1, SBC-4, and SBC-7. Comparing the new analogues with the mother compound, ADM, in terms of IC50 value, ME2303 was superior to, and KRN8602 was comparable to ADM, but SM5887 was inferior to the mother compound. Regarding the RAA, ME2303 was again the most potent agent, followed by ADM and SM5887. KRN8602 was less potent than SM5887 for all the cell lines except SBC-7, an SCLC cell line. As for the active metabolites of the new analogues, the RAA of ME2303-M-1 and SM5887-13-OH was almost comparable to that of ME2303 and SM5887, respectively, however, KRN8602-M2 was less potent than KRN8602.

Comparison of cross-resistance of new anthracyclines and their metabolites to ADM. The extent of cross-resistance of the newly developed anthracyclines to ADM, the mother compound, was investigated using an adriamycin-resistant small cell lung cancer subline, SBC-3/ADM100 and an etoposide-resistant subline, SBC-3/ETP which has a substantial resistance to ADM. Table 4 lists the IC50 values of the new analogues for SBC-3, SBC-3/ADM100, and SBC-3/ETP, and the extent of resistance of the new analogues to ADM. The extent of resistance was expressed as a relative resistance value which signifies the ratio of the IC50 for the resistant sublines to that for the parent cell line. As the result, SBC-3/ADM100 which is 106-fold more resistant to ADM than the parent SBC-3 showed less resistance to the new anthracyclines: 1.80-fold to KRN8602, 3.80-fold to SM5887 and 8.60-fold to ME2303. SBC-3/ETP which was 52.1-fold more resistant to ETP and 39.5-fold more resistant to ADM (15) were also less resistant to the analogues: 3.27-fold to KRN8602, 9.07-fold to SM5887 and 17.3-fold to ME2303. These findings are summarized as follows; the extent of cross-resistance to ADM was least for KRN8602, followed by SM5887 and ME2303.

As for the active metabolites of the new analogues, KRN8602-M2 and SM5887-13-OH were much more cross-resistant to ADM than KRN8602 and SM5887, while the extent of cross-resistance of ME2303-M-1 to ADM was almost comparable for that of the unchanged compound, ME2303.

Table 3 Comparison of the antitumor activity of adriamycin and new anthracycline analogues against human lung cance cell lines.

Drugs	Mean IC50 value (nM)±SD					
	SBC-2	SBC-3	SBC-4	SBC-7	ABC-1	EBC-1
Adriamycin	142±17.4 (0.0173)	18.8±4.50 (0.131)	185±37.8 (0.0133)	200±70.2 (0.0123)	51.0±15.4 (0.0482)	175±105 (0.0141)
ME2303	42.5±6.48 (0.115)	13.4±6.33 (0.363)	156±30.2 (0.0312)	169±139 (0.0288)	39.7±18.2 (0.123)	90.0±4.38 (0.0541)
ME2303-M-1	61.5±12.0 (0.0928)	14.7±3.53 (0.388)	188±10.6 (0.0304)	318±123 (0.0179)	117±8.39 (0.0488)	236±11.1 (0.0242)
KRN8602	123±0.424 (0.00622)	59.7±20.9 (0.0128)	346±68.0 (0.00221)	346±230 (0.00221)	81.8±33.9 (0.00935)	258±10.7 (0.00296)
KRN8602-M2	107±69.6 (0.000480)	8.04±3.36 (0.00639)	130±8.66 (0.000395)	367±180 (0.000140)	24.0±1.40 (0.00214)	144±4.18 (0.000357)
SM5887	827±86.9 (0.0153)	723±270 (0.0175)	3290±937 (0.00384)	6990±3350 (0.00181)	703±84.7 (0.0180)	2040±164 (0.00619)
SM5887-13-OH	132±15.2 (0.0100)	112±36.3 (0.0118)	279±66.0 (0.00474)	745±43.6 (0.00178)	48.7±14.2 (0.0272)	525±69.0 (0.00252)

IC50: 50% inhibitory concentration

SD: standard deviation

Numbers in parentheses are the value of AUC (μM•hour)/mean IC50 of each drug which represents relative antitumor activity. The AUC (μM•hour) is 2.46 for adriamycin, 4.87 for ME2303, 5.71 for ME2303-M-1, 0.765 for KRN8602, 0.0514 for KRN8602-M2, 12.6 for SM5887, and 1.32 for SM5887-13-OH.

Table 4 Comparison of the IC50 values and the relative resistance values of new anthracycline analogues in SBC-3, SBC-3/ADM100, and SBC-3/ETP.

Drugs	SBC-3	SBC-3/ADM100		SBC-3/ETP	
	IC50 value (nM)±SD	IC50 value (nM)±SD	Relative resistance value	IC50 value (nM)±SD	Relative resistance value
Adriamycin	18.8±4.50	1930±383	106±25.3	1090±246	39.5±7.36
ME2303	13.4±6.33	110±0.00	8.60±4.00	192±29.3	17.3±1.36
ME2303-M-1	14.7±3.53	264±46.0	18.3±0.550	178±33.2	11.0±3.32
KRN8602	59.7±20.9	77.5±17.1	1.80±0.600	260±60.0	3.27±0.420
KRN8602-M2	8.04±3.36	267±127	31.9±9.18	127±94.4	16.4±6.11
SM5887	723±270	3500±2090	3.80±0.900	4900±300	9.07±2.97
SM5887-13-OH	112±36.3	6930±231	59.5±22.1	4570±839	50.9±20.9

Relative resistance represents the value of IC50 for the resistant cell line/IC50 for the parent cell line of each drug.

Discussion

Synthesis of analogues from known active compounds has yielded new agents with increased antitumor activity, different antitumor spectra, decreased toxicity, or lack of cross-resistance with the mother compound. The toxicity that ultimately limits the use of ADM is linked to the development of irreversible cardiomyopathy. Synthesis of new anthracyclines lacking cardiotoxicity has been of particular interest to many investigators and new anthracycline analogues are reported to be less cardiotoxic (21, 22, 23).

If the clinical response of a specified tumor to a new agent could be assessed *in vitro* prior to

phase II clinical trials, time consuming, and sometimes risky, clinical trials could be minimized in the future. This study attempts to evaluate the antitumor activity of several newly developed anthracycline analogues by MTT assay using human lung cancer cell lines. In general, when predicting the clinical response to a certain cytotoxic agent on the basis of *in vitro* sensitivity testing, the antitumor activity involving the pharmacokinetic data, *i. e.*, the ratio of the peak plasma concentration (PPC) or the AUC *in vivo* to the IC50 *in vitro*, is often used as a parameter. The methods, however, still remain controversial. Horiuchi *et al.* (24) compared the antitumor activity of mitomycin C (MMC) analogues with that of MMC using the ratio of the PPC to the IC50 *in vitro*. A variety of new analogues was also evaluated using the PPC/IC50 ratio by Ohe *et al.* (25). However, the PPC may vary according to the modes of administration or the pharmacokinetics of the drugs. Sasaki *et al.* (26) suggested that the peak achievable concentration may be important in case of drug-resistant tumors such as NSCLC, whereas, in case of drug-sensitive tumors like SCLC, the AUC may be as valuable as the PPC. Meyers reported that the antitumor activity of ADM was proportional to the AUC, not to the PPC (27). Taking these findings into consideration, we adopted the ratio of the AUC to the IC50 to predict the clinical efficacy of the analogues.

ME2303 showed a higher antitumor activity than the mother compound, ADM, while KRN8602 and SM5887 were less active than ADM. Concerning the active metabolites of these analogues, SM5887 is transformed into SM5887-13-OH *in vivo* which showed much higher activity in terms of the IC50 (19). The RAA of the metabolite, however, is actually comparable for SM5887, because the AUC of the metabolite was much smaller than that of SM5887. Similarly, although ME2303-M-1 and KRN8602-M2 are as potent as their unchanged compounds in terms of the IC50, the RAA of these metabolites were lower than those of the respective unchanged compounds. Based on these results, it may be concluded that ME2303 is the most potent agent among anthracyclines presently available in the treatment of lung cancer.

While SBC-3/ADM100 and SBC-3/ETP overexpressing P-glycoprotein and being resistant to a wide variety of drugs, ME2303, KRN8602, and SM5887 were substantially less cross-resistant than the mother compound, ADM. Among those, KRN8602 showed significant activity in the drug-resistant sublines which were 40 to 100-fold more resistant to ADM. KRN8602-M2, the active metabolite of the agent, was much more cross-resistant to ADM in terms of the relative resistance value, while the unchanged compound, KRN8602, showed the least cross-resistance to ADM. The dissociated findings could be accounted for by a difference in the susceptibility to the drug-efflux mechanism in cells expressing MDR (14). From the clinical standpoint, however, the dissociation would not make much sense because the AUC of KRN8602-M2 was much smaller than that of KRN8602.

Ruben *et al.* (28) evaluated the MTT assay for a large-scale screening of anticancer agents by determining the IC50s as a parameter. Monks *et al.* (29) also implemented a new investigational, *in vitro*, disease-oriented, drug discovery screening test. In this setting, a screening system for anticancer drugs using human cancer cell lines has been developed. The establishment of a reliable drug screening system will become much more important in the future to avoid fruitless phase II clinical trials for newly developed anticancer agents. The clinician may be ethically obligated to use a standard therapy, and subsequently a second-line treatment, if available. Only a patient who has failed the standard chemotherapy becomes a candidate for phase II trials in which the activity of a new drug is evaluated. In the case of SCLC, however, second-line chemotherapy will fail in producing a major clinical response or in prolonging survival. A number of well-characterized cell lines derived from both untreat-

ed and treated human tumors, and acquired multidrug resistant sublines, as well, would provide useful tools for the screening of new drugs (30). The model described here to predict antitumor activity of new agents should be verified by the results of phase II studies currently underway.

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