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Research paper

Antitumor activity of KW-2170, a novel pyrazoloacridone derivative

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5-(3-Aminopropyl)amino-7,10-dihydroxy-2-(2-hydroxethyl)-aminoethyl-6H-pyrazolo[4,5,1-*de*]acridin-6-one dihydroxychloride (KW-2170), a novel derivative of pyrazoloacridone, was selected and evaluated for its antitumor activity and toxicity in mice. KW-2170 exhibited antitumor activity superior to adriamycin (ADM) against Sarcoma 180, breast carcinoma MM102 and fibrosarcoma Meth A inoculated s.c. in mice. Its therapeutic index (LD_{10}/ED_{50}) was higher than that of ADM on two murine carcinoma models, MM102 and Meth A. KW-2170 showed significant antitumor activity against 17 human tumor xenografts of a total of 24 tumors tested and the total tumor response rate by treatment with KW-2170 was significantly higher than that by ADM (70.8 versus 58.3%). In particular, human lung carcinoma was highly sensitive to KW-2170, and a marked tumor regression was observed on Lu-65 and Lu-99 human lung carcinoma xenograft models. Ovary and pancreas carcinomas were also sensitive to the drug. Additionally, its therapeutic index was also high on these human carcinoma models in comparison with that of ADM. The best antitumor efficacy of KW-2170 was observed by a weekly treatment schedule followed by a single treatment schedule and a successive administration schedule also tended to be toxic to the hosts. KW-2170 exhibited very low cross-resistance against four lines of multidrug resistant tumors expressing high levels of P-glycoprotein, and the drug showed significant antitumor activity against ADM-resistant human ovary carcinoma A2780/ADM and against nasopharynx carcinoma KB-A1 xenografts which were not sensitive to ADM. These results indicate that KW-2170 has a very potent antitumor activity and is feasible as a new antitumor drug against ADM-refractory solid tumors in clinics. [© 1998 Rapid Science Ltd.]

Key words: Adriamycin, antitumor, KW-2170, mouse.

Introduction

Adriamycin (ADM), a potent DNA intercalator, has good antitumor activity against a broad spectrum of experimental tumors and is used as one of the key drugs for chemotherapy of human neoplastic disease.[1] However, its clinical use has often been limited by either severe dose-related cardiotoxicity or development of drug resistance.[2,3] For these reasons, the effort to search for new anthracyclines or other types of DNA intercalators without cardiotoxicity and with superior antitumor activity has continued. In that context, an anthrapyrazole derivative, CI-941, has been developed as a novel class of synthetic DNA intercalative agent with a broad antitumor spectrum against experimental tumors[4] and is highly active in the treatment of advanced breast cancer in early phase clinical studies.[5]

We synthesized a variety of derivatives of pyrazo-loacridone with DNA intercalating structure and with antitumor activity. Among them, we selected 5-(3-aminopropyl)amino-7,10-dihydroxy-2-(2-hydroxyethyl)-aminomethyl-6H-pyrazolo[4,5,1-*de*]acridine-6-one dihydroxychloride (KW-2170) (Figure 1)[7] as a candidate which possesses excellent antitumor activities *in vitro* and *in vivo*, and very low cardiotoxicity in animal studies. In preliminary experiments, KW-2170 showed potent antitumor activity against Sarcoma 180 and P388 leukemia, and was effective against murine multidrug resistant cells P388/ADM *in vivo*.[7] In addition, KW-2170 showed marked DNA intercalating ability in an ethidium bromide fluorescence assay and marked antiproliferative activity against human uterine carcinoma HeLa S_3 cells.[7] In acute or sub-acute cardiotoxicity studies, KW-2170 was found to be significantly less cardiotoxic than ADM in hamster and dog models.

In the present study, the precise antitumor activity of KW-2170 was examined against various murine and human tumor models, including some ADM-resistant human tumors, and was compared with ADM. The optimal treatment schedule was also examined by the i.v. administration route.

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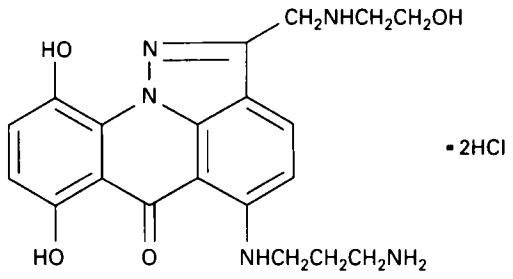

Figure 1. Structure of KW-2170.

Materials and methods

Chemicals

KW-2170, ADM and CI-941 were prepared by Kyowa Hakko Kogyo (Tokyo, Japan) and etoposide (VP-16) was purchased from Nihon Kayaku (Toyko, Japan). These drugs were dissolved and diluted in sterile 0.9% NaCl solution (Ohtsuka Pharmaceutical, Tokyo, Japan) just prior to their use.

Animals and tumors

Male ddY mice, C57BL/6 mice, BALB/c mice and female C3H/He mice were obtained from Japan SLC (Shizuoka, Japan). Male nude mice (BALB/c-*nu/nu*, 5-7 weeks old) weighing 20-25 g were obtained from Nippon Clea (Tokyo, Japan). They were housed in a separate experimental room, and given sterilized food and water *ad libitum*. All animal experiments were conducted using five mice in a group except for the lethal toxicity study.

Murine fibrosarcoma Meth A was provided by Dr H Sasaki (Sasaki Laboratory, Tokyo, Japan), murine breast carcinoma MM102 was provided by Dr T Tachibana (Tohoku University, Miyagi, Japan) and Sarcoma 180 was provided by National Cancer Center (Tokyo, Japan).

Human large cell lung carcinoma Lu-65, human breast carcinoma MX-1 and human stomach carcinoma 4-1ST were provided by Dr Y Ohnishi (Central Institute for Experimental Animals, Kanagawa, Japan). Human large cell lung carcinoma Lu-99 cells was provided by Dr S Hirohashi (National Cancer Center), human colon adenocarcinoma DLD-1 (ATCC, through Dainippon Pharmaceutical, Japan) human pancreas adenocarcinoma PSN-1 were provided by Dr K Yamaguchi (National Cancer Center), human ovary carcinoma A2780 and ADM-induced resistant lines A2780/ADM[8] were provided by Dr T Tsuruo (Tokyo University, Tokyo, Japan); human nasopharynx carcinoma KB and its ADM-induced resistant lines KB-A1[9] were provided by Dr K Ueda (Kyoto University).

These tumor cell lines were established by inoculating the cells from each cell line cultured s.c. into nude mice.

Evaluation of antitumor activity

Sarcoma 180, MM102 and Meth A cells were inoculated s.c. at the axillary regions of the ddY strain of mice and C3H/He and BALB/c strains of mice, respectively. Other solid tumors were innoculated into the flank of BALB/c-*nu/nu* mice. To evaluate the antitumor activity against s.c. inoculated tumors, the tumor volume was calculated based on the following formula according to the method of the National Cancer Institute[10] after measuring the lengths and widths of the tumors:

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

Efficacy of drugs against human tumors inoculated into nude mice 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 treatment. The T/C value was calculated by the mean V/V_0 value of the treated group versus that of the untreated group.

Antiproliferative activity

Human myelogenous leukemia K562 and its ADM-induced resistant lines K562/ADM[11] were provided by Dr T Tsuruo, human nasopharynx carcinoma KB and its etoposide (VP-16)-induced resistant line KB/VP-2[12] or teniposide (VM-26)-induced resistant line KB/VM-4[13] were provided by Dr M Kuwano (Kyushu University, Fukuoka, Japan), and human breast carcinoma MCF-7 and its ADM-induced resistant lines MCF-7/ADM[14] were provided by Dr K H Cowan (National Cancer Institute). Descriptions of the establishment and characterization of all cell lines are in preparation.[8,9,11-14] The cells were precultured for 24 h in 96-well microplates (Nunc, Roskilde, Denmark) containing 0.1 ml of culture medium at 37° C in a humidified atmosphere containing 5% CO_2 in air. Then cells were treated with each compound for 72 h. The antiproliferative activity of each compound was evaluated by a cell count method using a Microcell Counter (Toa Medical Electronics, Hyogo, Japan). The IC_{50} (compound concentration causing a 50% inhibition of cell

growth) was calculated by regression line of dose response. The degree of resistance (RD) for test compounds was calculated from the ratio of IC_{50} between the parent and its resistant lines.

Detection of P-glycoprotein expression by flow cytometry analysis

Expression of P-glycoprotein on the cells was detected by direct immunofluorescence assay with anti-P-glycoprotein monoclonal antibody, MRK16 (Kyowa Medex, Tokyo, Japan). To avoid a false-positive reaction of MRK16 with the Fc receptor, the $F(ab')_2$ fragment of the monoclonal antibody was used for the assay. Cells were harvested and washed with Hank's solution containing 10% heat-inactivated fetal bovine serum (FBS; Gibco, Grand Island, NY) and 0.1% sodium azid (Sigma, St Louis, MO). The cells were incubated for 30 min 4°C with 50 µl of FITC-labeled MRK16 $F(ab')_2$ fragment (at 10 µg/ml) or 20 µl of FITC-labeled IgG $F(ab')_2$ fragment (Cappel, Durham, NC) as a negative control. After washing the cells were analyzed by a flow cytometer (Epics Elite; Coulter, Hialeah) and the level of P-glycoprotein expression was calculated by the IMMUNO-4 program.

Toxicity

Lethal toxicity of test compounds in mice was observed for 30 days after administration of the compounds and LD_{10} (10% lethal dose) or LD_{50} (50% lethal dose) values were calculated by the probit method. The body weight of the mice was measured by an electronic balance FX-300 (A & D, Tokyo, Japan).

Table 1. Antitumor activity of KW-2170 and ADM against murine solid tumors

Tumor	Compound	Dose (mg/kg)	Tumor volume (mm^3, mean ± SD)	T/C (%)	On day
Sarcoma 180	untreated	0	2386 ± 52	100	7
	KW-2170	8.4	368 ± 64[a,b]	15	7
	ADM	14	1247 ± 203	52	7
MM102	untreated	0	1396 ± 94	100	14
	KW-2170	8.4	240 ± 107[a,b]	17	14
	ADM	14	865 ± 266	62	14
Meth A	untreated	0	1244 ± 150	100	9
	KW-2170	8.4	58 ± 21[a]	5.0	9
	ADM	14	52 ± 25[a]	4.0	9

Sarcoma 180 (5×10^6/mouse), MM102 breast carcinoma (1×10^6/mouse) and Meth A fibrosarcoma (1×10^6/mouse) were innoculated s.c. on day 0.
Compounds were administered i.v. on day 1.
[a]T/C (%) ≤ 50 and $p < 0.05$ by Mann–Whitney's rank-sum test as compared with the untreated group.
[b]$p < 0.05$ by Mann–Whitney's rank-sum test as compared with the ADM-treated group.

Table 2. Therapeutic index of antitumor activity of KW-2170 and ADM against murine and human solid carcinomas

Species	Tumors	KW-2170			ADM		
		T/C (%)	ED_{50} (mg/kg)	TI[a]	T/C (%)	ED_{50} (mg/kg)	TI
Murine[b]	Sarcoma 180	15[b]	2.2	3.8	52	14	< 1.0
	MM102	17[d]	1.5	5.6	62	14	< 1.0
	Meth A	5.0[d]	< 1.7	> 4.9	4.0[d]	3.0	4.5
Human[c]	Lu-65	1.0[e]	0.5	17	5.7[e]	4.4	3.1
	Lu-99	2.5[e]	1.1	7.6	49[e]	13	1.0
	MX-1	13[e]	2.0	4.2	36[e]	11	1.2

[a]Therapeutic index calculated as the ratio of MTD to ED_{50}.
[b]Tumor cells were innoculated s.c. into mice on day 0 as described in Table 1. KW-2170 and ADM were administered i.v. on day 1.
[c]Tumor cells (8 mm^3 fragments) were innoculated s.c. into BALB/c-*nu/nu* mice. When tumors had grown to a size of between 50 and 300 mm^3 (day 0), KW-2170 and ADM were administered i.v.
[d]T/C (%) ≤ 50 and $p < 0.05$ by Mann–Whitney's rank-sum test as compared with the untreated group.
[e]T/C (%) ≤ 50 and $p < 0.01$ (one-sided) by Mann–Whitney's rank-sum test as compared with the untreated group.

Statistical analysis

The analysis of the SAS statistical program was conducted using the Mann-Whitney rank-sum test.

Results

Antitumor activity of KW-2170 against murine solid tumors

The antitumor activity of KW-2170 against murine solid tumors was examined and compared with that of ADM, and their antitumor activities at the respective LD_{10} (KW-2170, 8.4 mg/kg; ADM, 14 mg/kg) are shown in Table 1. KW-2170 exhibited statistically significant antitumor activity against all tumors tested in this experiment. The antitumor activity of KW-2170 was clearly greater than that of ADM against Sarcoma 180 and MM102 (Table 1). KW-2170 exhibited a marked antitumor activity against Meth A fibrosarcoma and ADM also showed comparative antitumor activity at the LD_{10} dose. Therapeutic indexes (TI) calculated as the ratio of LD_{10} to ED_{50} (the estimating dose showing T/C=50 by a regression analysis) of KW-2170 were higher

Table 3. Spectrum of antitumor activity of KW-2170 against human carcinomas inoculated into nude mice

Origin	n	Antitumor activity[a]	
		KW-2170	ADM
Lung			
(NSLC)	6	4/6[b]	4/6
(SCLC)	1	1/1	0/1
Ovary	3	3/3	2/3
Pancreas	3	2/3	1/3
Breast	3	1/3	1/3
Colon	5	2/5	2/5
Prostate	1	1/1	0/1
Stomach	1	1/1	1/1
Hepatoma	1	1/1	1/1
Total	24	17/24 (70.8)[c]	14/24 (58.3)

Tumor cells (8 mm^3 fragments) were inoculated s.c. into BALB/c-*nu/nu* mice. When tumors had grown to a size of between 50 and 300 mm^3 (day 0), KW-2170 (8.4 mg/kg) or ADM (14 mg/kg) were administered i.v.
[a]T/C (%) $\leq$ 50 and $p < 0.01$ (one-sided) by Mann–Whitney's rank-sum test as compared with the untreated group.
[b]Number of shows positive response in total xenografts tested.
[c]Response rate (%).

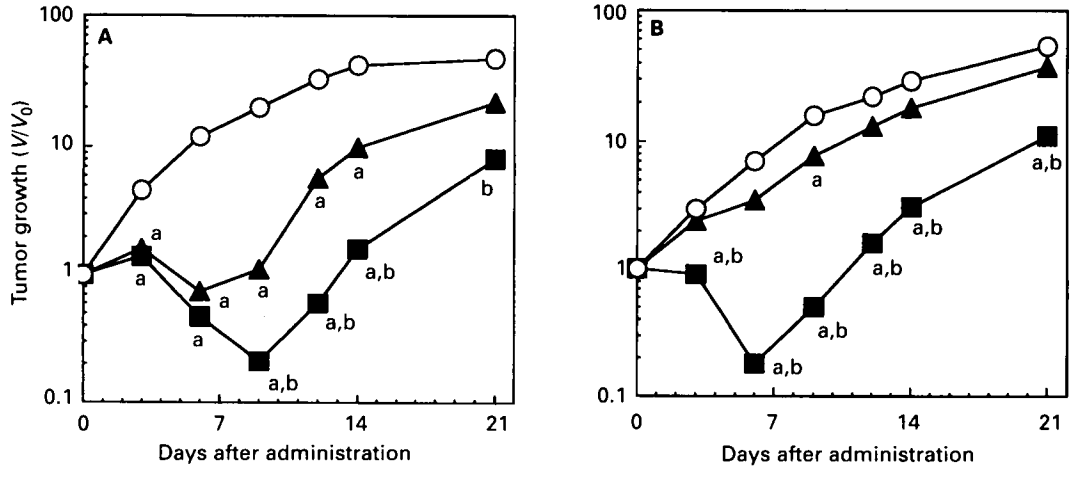

Figure 2. Tumor growth patterns of Lu-65 (A) or Lu-99 (B) inoculated into nude mice. KW-2170 (■; 8.4 mg/kg) or ADM (▲; 14 mg/kg) was administered i.v. on day 0. Untreated (○). $p < 0.01$ by Mann–Whitney's rank-sum test as compared with the untreated group (a) or $p < 0.05$ by Mann–Whitney's rank-sum test as compared with the ADM-treated group (b).

than that of ADM on three murine solid tumors (Table 2).

Antitumor activity against human solid tumors

The antitumor activity of KW-2170 was further evaluated using various human solid tumors (total 24 tumor lines) inoculated into nude mice (Table 3).

The efficacy of KW-2170 and that of ADM were compared by single i.v. administration at their LD_{10}, because the body weight loss in host mice by KW-2170 or ADM treatment was similar at this dose (data not shown). Antitumor activity of KW-2170 against human xenografts was expressed as positive response rate which means antitumor activity significantly more than 50% growth inhibition (Table 3). KW-2170 showed a marked and broad antitumor activity against 17 human tumors of 24 tumors tested. The efficacy (response) rate of KW-2170 (70.8%) was significantly higher than that of ADM (58.3%). Human non-small cell lung, ovary and pancreatic carcinomas tended to be more sensitive to KW-2170. Large cell carcinoma Lu-65 and Lu-99 was very sensitive to KW-2170, and marked suppression of tumor growth was observed at 10–14 days after the drug treatment with T/C values less than 10% as shown in Figure 2. The antitumor activity of KW-2170 was significantly superior to that of ADM and TI values of KW-2170 on these tumors were clearly higher than those of ADM (Table 2).

Schedule dependency of antitumor activity of KW-2170

The schedule dependency of antitumor activity of KW-2170 by three different protocols was examined against human large cell lung carcinoma Lu-65-bearing mice (Figure 3). KW-2170 exhibited a very potent antitumor activity against Lu-65 by a weekly and/or a single treatment schedule, both of which were more potent compared with that of a consecutive treatment schedule (Table 4). It may be that the weekly administration schedule was suitable for KW-2170 because of its wide therapeutic window without mortality or toxicity estimated by body weight loss (data not shown). In another experiment, KW-2170 was confirmed to be more potent in antitumor activity by the weekly schedule (days 0, 7 and 14) against human colon carcinoma DLD-1, human pancreatic carcinoma PSN-1 and human stomach carinoma (Table 5). Additionally, the myelotoxicity of KW-2170 estimated by the counts of peripheral blood cells and bone marrow cells was less in the weekly treatment schedule as compared with that in the single and the consecutive treatment schedules (data not shown). These data suggest that the weekly treatment schedule may be the most favorable one for KW-2170.

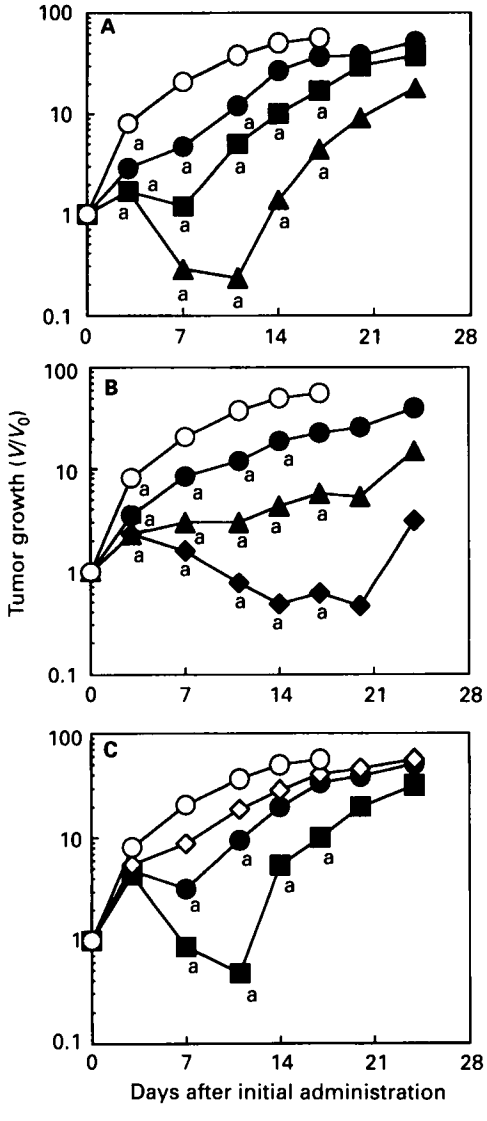

Figure 3. Tumor growth patterns of schedule dependency of human lung carcinoma Lu-65 inoculated into nude mice. KW-2170, single injection (●; 2.1 mg/kg), (■; 4.2 mg/kg) or (▲; 8.4 mg/kg) was administered i.v. on day 0 (A) and KW-2170, weekly injection (●; 1.4 mg/kg), (▲; 2.8 mg/kg) or (◆; 4.2 mg/kg) on days 0, 7 and 14 (B) and KW-2170, 5 successive injections (◇; 0.21 mg/kg), (●; 0.42 mg/kg) or (■; 0.84 mg/kg) days 0–4 (C). Untreated (○). $p<0.01$ by Mann–Whitney's rank-sum test as compared with the untreated group (a).

Table 4. Schedule dependency of antitumor activity of KW-2170 against human lung carcinoma Lu-65 inoculated into nude mice

Schedule (day)	Dose (mg/kg/day)	Total dose (mg/kg)	T/C (%)	Mortality
0	2.1	2.1	22[a]	0/5
0	4.2	4.2	5.6[a]	0/5
0	8.4	8.4	0.62[a]	0/5
0, 7, 14	0.70	2.1	50[a]	0/5
0, 7, 14	1.4	4.2	32[a]	0/5
0, 7, 14	2.8	8.4	8.0[a]	0/5
0, 7, 14	4.2	13	1.0[a]	0/5
0–4	0.21	1.1	42	0/5
0–4	0.42	2.1	15[a]	0/5
0–4	0.84	4.2	1.3[a]	0/5
0–4	1.7	8.4	0.038	2/5

Lu-65 cells (8 mm^3 fragments) were inoculated s.c. into BALB/c-*nu/nu* mice. When tumors had grown to a size of between 50 and 300 mm^3 (day 0), KW-2170 was administered i.v. following the schedule indicated in the table.
[a]T/C (%) ≤ 50 and $p < 0.01$ (one-sided) by Mann-Whitney's rank-sum test as compared with the untreated group.

Table 5. Schedule dependency of antitumor activity of KW-2170 and ADM against human carcinomas inoculated into nude mice

Compounds	Schedule (day)	Dose (mg/kg/day)	T/C (%)			
			Lu-65 (lung)	DLD-1 (colon)	PSN-1 (pancreas)	4-1ST (stomach)
KW-2170	0	8.4	0.62[a]	49[a]	36[a]	37[a]
	0, 7, 14	6.3	1.0[a]	28[a,b]	25[a,b]	16[a]
ADM	0	14	NT[c]	43	37[a]	30[a]
	0, 7, 14	10	NT	48	43[a]	26[a]

Tumor cells (8 mm^3 fragments) were inoculated s.c. into BALB/c-*nu/nu* mice. When tumors had grown to a size of between 50 and 300 mm^3 (day 0), KW-2170 or ADM were administered i.v. following the schedule indicated in the table.
[a]T/C (%) ≤ 50 and $p < 0.01$ (one-sided) by Mann–Whitney's rank-sum test as compared with the untreated group.
[b]$p < 0.05$ by Mann–Whitney's rank-sum test as compared with the ADM-treated group.
[c]Not tested.

Antitumor activity of KW-2170 against the various anti-cancer drug-resistant human tumor cell lines

P-glycoprotein levels in four ADM-induced, drug-resistant cell lines (K562/ADM, A2780/ADM, KB-A1 and MCF-7/ADM) and two epipodophyllotoxins-induced resistant lines (KB/VP-2 and KB/VM-4) were measured by flow cytometry in order to reveal the relationship between the expression level and chemosensitivity of the cell lines to KW-2170, CI-941 and ADM. All four ADM-induced, drug-resistant cells showed high levels of P-glycoprotein expression (Table 6 and Figure 4A–D), and two epipodophylotoxin-induced, drug-resistant cells showed low levels of P-glycoprotein expression (Table 6).

Four ADM-induced resistant cell lines but not MCF-7/ADM showed less cross-resistance to KW-2170 compared with that to ADM and CI-941, both of which were more than 50 times the cross-resistance ratio (Table 6). In the case of MCF-7/ADM, however, the degree of resistance to KW-2170 recorded note for ADM and CI-941. To elucidate whether the low cross-resistance property of KW-2170 *in vitro* is therapeutically beneficial, an *in vivo* evaluation of KW-2170 against A2780/ADM and KB-A1 cells inoculated in nude mice was conducted (Figure 5A and B). ADM did not show any significant inhibitory activity on the growth of these tumors, indicating that these cell lines retained their ADM-resistant potencies even after *in vivo* passages. On the contrary, KW-2170 exhibited a statistically significant antitumor activity against both tumors examined in this experiment. Thus, the superior activity of KW-2170 to ADM was confirmed on A2780/ADM and KB-A1 tumor xenograft models *in vivo*.

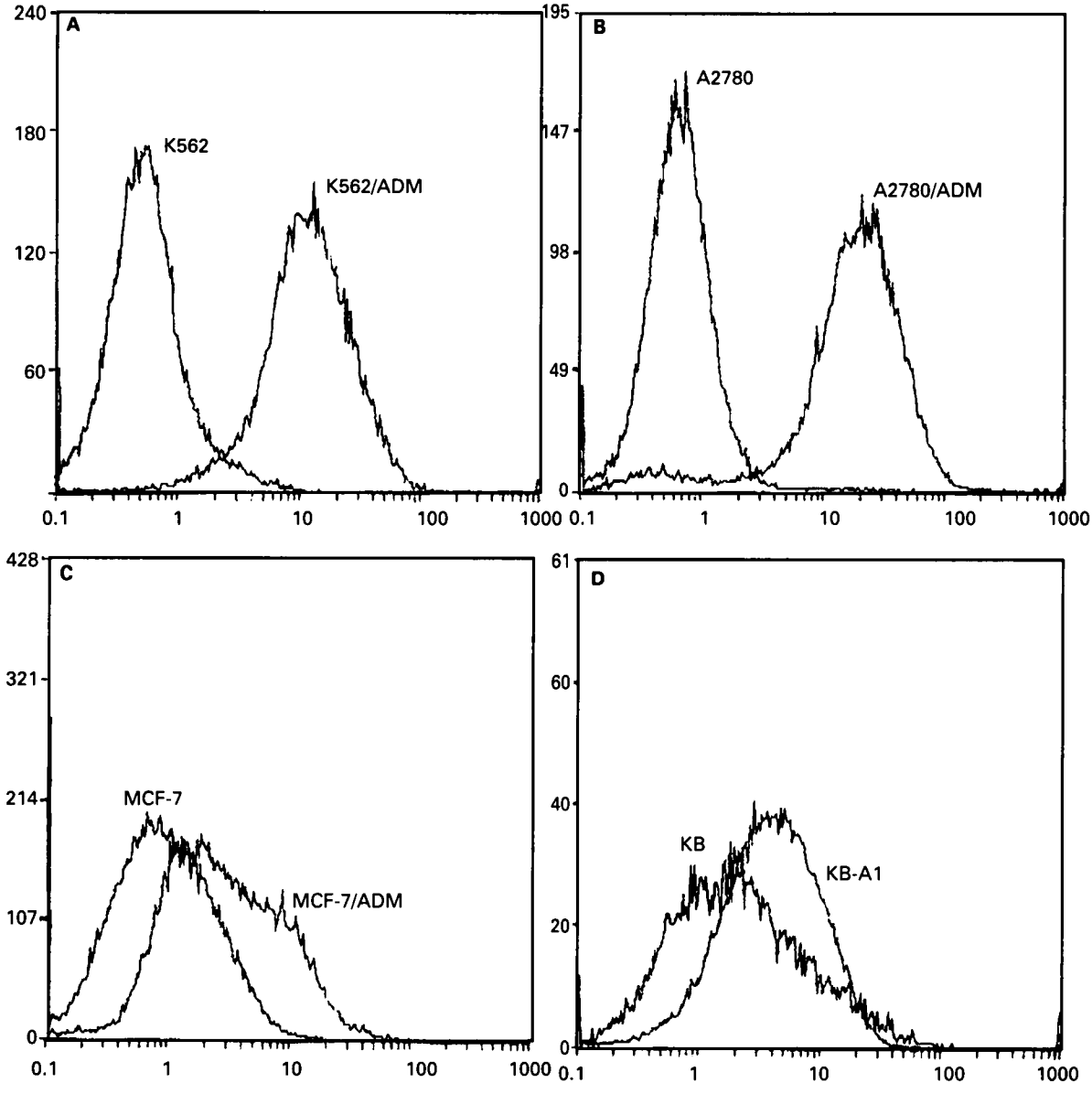

Figure 4. Flow cytometric analysis of P-glycoprotein expression in *MDR* cell lines. In each overlay histogram, the horizontal axis records FITC fluorescence of MRK16-treated or isotype control cells, while the vertical axis represents number of cells. (A) K562/ADM, (B) A2780/ADM, (C) MCF-7/ADM and (D) KB-A1.

Discussion

In the present study, we evaluated the antitumor activity of KW-2170, a new pyrazoloacridone derivative, compared with ADM, the most widely used anthracycline in clinics. The antitumor efficacy of KW-2170 against 24 human tumor xenograft models implanted into nude mice was superior to that of ADM. In particular, KW-2170 exhibited a marked antitumor effect against human non-small cell lung carcinomas Lu-65 and Lu-99 *in vivo*. The reason why KW-2170 showed superior antitumor activity to ADM could be explained in part by its greater lipophilicity and its higher intracellular accumulation compared with that of ADM (data not shown).

One of the remarkable features of the antitumor effect of KW-2170 was its favorable property of low cross-resistance against multidrug resistant (*MDR*) lines of human tumors (K562/ADM, A2780/ADM, MCF-7 and KB-A1) which were confirmed to express high levels of P-glycoprotein. In addition, this drug exhibited significant antitumor activity against ADM-

Table 6. Antiproliferative activity of KW-2170 and ADM against drug-resistant human tumor cell lines

Cell lines	Expression of P-glycoprotein (%)[b]	Degree of resistance (DR)[a]			
		KW-2170	ADM	CI-941	PD[c]
K562/ADM	97.3	±[d]	+	++	
A2780/ADM	92.0	±	++	++	
KB-A1	73.8	±	++	++	
MCF-7/ADM	69.4	++	+++	+++	
KB/VP-2	−26.2	±	±	+	++
KB/VM-4	−26.2	±	±	+	++

Tumor cells ($2–5 \times 10^4$/well) were cultured on day 0 and treated with compounds on day 1 for 72 h. On day 4, the antiproliferative activity was determined by the cell count method and the degree of resistance was calculated.
[a]Degree of resistance was calculated as IC_{50} for resistant cells/IC_{50} for sensitive cells.
[b]By flow cytometry analysis as described in Materials and methods.
[c]Parent drug which induced the resistance.
[d]Scoring criteria: ±, DR > 1.0–10; +, > 11–50; ++, > 51–500; +++, > 501.

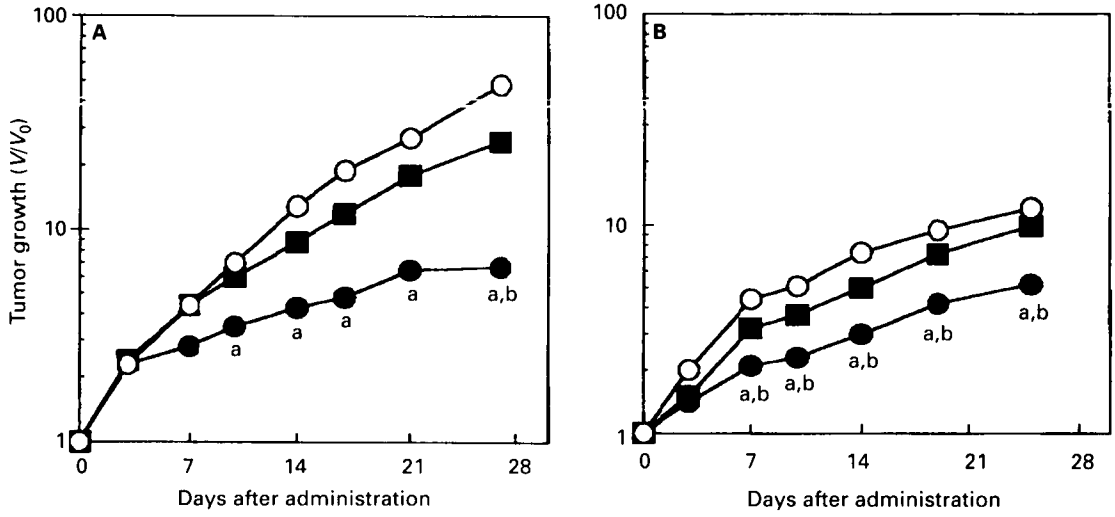

Figure 5. Tumor growth patterns of ADM-resistant ovary carcinoma A2780/ADM (A) or nasopharynx carcinoma KB-A1 (B) inoculated into nude mice. KW-2170 (●; 8.4 mg/kg) or ADM (■; 14 mg/kg) was administered i.v. on day 0. Untreated (○). $p < 0.01$ by Mann–Whitney's rank-sum test as compared with the untreated group (a) or $p < 0.05$ by Mann–Whitney's rank-sum test as compared with the ADM-treated group (b).

resistant human ovary carcinoma A2780/ADM and nasopharynx carcinoma KB-A1 xenografts in nude mice. On the other hand, CI-941, which is similar in its formula but different in its chemical properties from KW-2170,[7] showed cross-resistance to *MDR*-expressing cells[4] and anthracycline-resistant P388/ADM cells.[15] This resistance of CI-941 was partially reversed by a calcium channel blocker, verapamil, in P388/ADM[15] and various P-glycoprotein-expressing human carcinoma cell lines (data not shown).

In the other experiments, the intracellular accumulation and retention of KW-2170 in A2780 and A2780/ADM were confirmed to be greater than those of ADM (data not shown). Thus, it could be concluded that KW-2170 is more potent in its antitumor efficacy than ADM and also more potent than CI-941 against some MDR cells expressing high levels of P-glycoprotein. These properties suggest that KW-2170 can be expected to show efficacy against some ADM refractory solid tumors in clinics.

KW-2170 has a potency to inhibit topoisomerase II enzyme by stabilization of a topoisomerase II-DNA cleavable complex, leading to double-strand DNA cleavage (data not shown). Interestingly, KW-2170 also shows antiproliferative activity against etoposide-resistant KB/VP-2 cells[16] and teniposide-resistant KB/VM-4 cells,[16] the former of which were confirmed to decrease in enzyme activity of topoisomerase IIα and

the latter were confirmed to express high levels of *MRP* (multidrug resistant-associated protein)[17] with as high sensitivity as shown against their parent cells, respectively. Recent evidences indicate that KW-2170 was not reduced by enzymes of rat liver linking a redox cycle as a substrate for the enzymes *in vitro*.[18] By contrast, ADM was readily reduced under identical conditions and clearly formed a drug-free radical signal.[18,19] These results also suggest that the action mechanisms of KW-2170 are different from those of ADM and are potentially less cardiotoxic. In the present study, KW-2170 provided very potent antitumor activity against various murine and human xenograft tumor models, and it was superior to ADM, although the true mechanism of action of KW-2170 superior to ADM remains to be elucidated.

Conclusion

KW-2170 exhibited growth-inhibitory activity against human non-small cell lung carcinomas and ADM-resistant carcinomas inoculated into nude mice, suggesting that the drug is an interesting candidate for further evaluation as a new antitumor agent.

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