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Comparative pharmacokinetics of KRN8602, a new morpholino anthracycline, and Adriamycin in tumor-bearing mice

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Abstract It has been reported that KRN8602 shows antitumor effects similar or superior to those of Adriamycin (ADM) against several murine and human cell lines and has been found to be effective against multidrug resistant tumor cells. We investigated the pharmacokinetics of KRN8602, a new morpholino anthracycline, in comparison with ADM in mice bearing colon26 adenocarcinoma. After intravenous administration, both drugs disappeared triexponentially from the plasma and KRN8602 was eliminated faster than ADM. The rate of elimination of KRN8602 from tissues was also faster than that of ADM. The relative order of the area under the curve (AUC) of KRN8602 was spleen > tumor > small intestine > lung > kidney > heart > liver > brain > plasma, while that of ADM was spleen > kidney > lung > liver > heart > small intestine > tumor > plasma. ADM was not detectable in the brain. The AUC of KRN8602 was higher than that of ADM in the tumor and brain, but it was lower in other tissues. The tissue-to-plasma concentration ratio (Kp_{app}) of KRN8602 was higher than that of ADM in the tumor, spleen, small intestine and brain. KRN8602 was metabolized to several metabolites. The concentrations of M1 and M2 (glycoside-type metabolites) was relatively high in the spleen. M3 (aglycone-type metabolite) showed a very high AUC ratio in the liver (34%). In tumor, M1 and M2 concentrations were low and M3 was not detected. KRN8602 had a greater activity than ADM and M2 had a cytotoxic activity similar to KRN8602 against colon26 cells in an MTT assay. These results suggest that the strong antitumor effect of KRN8602 against colon26 is due not only to its strong cytotoxic activity but also to its marked transferability into tumors. KRN8602 shows better selective toxicity than ADM, because KRN8602 is more selective for tumors than ADM and less is transferred to normal tissues.

Key words Pharmacokinetics · Morpholino anthracycline · Tumor-bearing mice

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Introduction

The anthracycline antitumor antibiotics such as Adriamycin (ADM) occupy an important position in the field of cancer chemotherapy [1, 3]. When treatment is prolonged, however, cardiotoxic effects [10] and multidrug resistance appear [17] and become serious therapeutic problems. In order to reduce these side effects and improve the therapeutic effect, a number of compounds have been newly synthesized and are now under development.

KRN8602 (MX2.HCl), 3′-deamino-3′-morpholino-13-deoxo-10-hydroxycarminomycin hydrochloride, is a new morpholino anthracycline which has a morpholino ring at the 3′-position of the amino sugar [18]. This compound shows a similar or superior antitumor activity compared to ADM against various experimental tumors [8, 18, 19] and was also effective against multidrug-resistant cells in vitro and in vivo [2, 6, 13, 18, 19]. Subacute cardiotoxicity is much weaker than for ADM. KRN8602 shows beneficial effects against cerebral tumors [7]. The structural formulas of KRN8602 and ADM are given in Fig. 1.

To further characterize the biological properties of this analogue, we studied its pharmacokinetics in tumor-bearing mice and compared them with those of ADM administered at equal doses. We also evaluated the antitumor activities of KRN8602, its metabolites and ADM using an MTT assay.

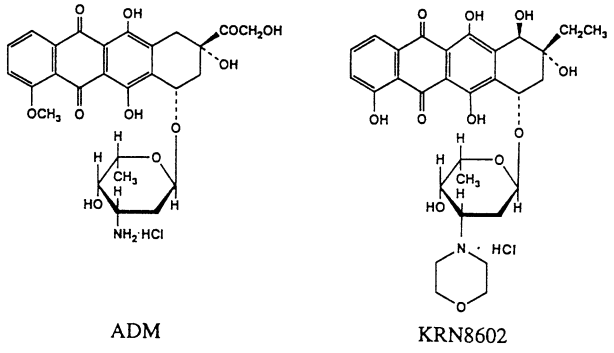

Fig. 1 Structures of KRN8602 and Adriamycin

Materials and methods

Drugs

KRN8602 and authentic samples of its metabolites used in this study were synthesized by Kirin Brewery Co. (Tokyo, Japan). Adriamycinone (AON) and daunomycinone (DON) were prepared from ADM and daunomycin (DM) in our laboratory. ADM (doxorubicin hydrochloride) and DM (daunorubicin hydrochloride) were purchased from Kyowa Hakko Kogyo Co. (Tokyo, Japan) and Meiji Seika Co. (Tokyo, Japan), respectively. Aqueous drug solutions were freshly prepared immediately before use. Two mg/kg KRN8602 or ADM were injected intravenously (i.v.) in a volume of 10 ml/kg body weight.

Animals and tumor

Female CDF1 mice were obtained from Japan SLC Co. (Shizuoka, Japan). The animals were maintained under standard laboratory conditions. They received subcutaneous transplants of colon26 adenocarcinoma into the right flank at 6 weeks of age were maintained for an additional 2 weeks. Of these animals, those with tumors that reached a long diameter of about 1 cm were used for pharmacokinetic study. At the time of administration, tumor-bearing animals weighed 22.4 ± 1.1 g for the KRN8602 group and 23.7 ± 0.9 g for the ADM group.

Administration of drugs and sample collection

KRN8602 or ADM was given i.v. to three tumor-bearing mice per time point and blood was collected into heparinized tubes by severing the carotid artery 2, 5, 10, 15 and 30 min, and 1, 2, 4, 6, 8, 12, 24 and 48 h (and 96 h for ADM only) after administration. Each blood sample was centrifuged at 10 000 *g* for 2 min to obtain plasma. Equal volumes of plasma obtained from three animals per time point were mixed and stored at $-80°C$ until analyzed.

Tumor and tissues (liver, kidney, heart, lung, spleen, small intestine, and brain) were removed at 10 and 30 min, and 2, 4, 8, 24 and 48 h (and 96 h for ADM only), rinsed in cold saline, rapidly frozen on dry ice, and then stored at $-80°C$ until used. Extraction was performed within not more than 3 weeks of sample collection.

Determination of drugs

The concentrations of KRN8602 and its metabolites were measured according to the previously reported method [14]. An outline of the measurement procedure is as follows. Plasma was diluted 1 : 1 with 1% H_3PO_4 and the mixture (1 ml) was filtered through a Sep-pack C18 cartridge column (Waters Associates, Hilford, Mass., USA). After the column was washed with 2 ml 0.1% H_3PO_4 and 10 ml distilled water, elution was carried out with 2.5 ml CH_3OH and the eluate was evaporated to dryness under a stream of nitrogen. Tissues were homogenized in 20 m*M* KH_2PO_4/H_3PO_4 (pH 2.0) to obtain a 5% or 10% homogenate in accordance with the tissue weight. The homogenate was diluted 1 : 1 with 80% CH_3CN containing 10 m*M* $MgCl_2$ and vigorously stirred, and the mixture was then centrifuged at 10 000 *g* for 5 min. To 1 ml of the supernatant, 1 ml 0.2 *M* K-phosphate buffer (pH 8.0) and 8 ml $CHCl_3/CH_3OH$ (4 : 1) were added and the mixture was shaken for 10 min. After a 10-min centrifugation at 1100 *g*, the organic layer was collected and evaporated.

To the extract of plasma and tissues, 0.5 ml 0.2 *M* citrate buffer (pH 2.2) and 2.5 ml $(C_2H_5)_2O$ were added. The solution was then stirred and centrifuged, and the organic layer (aglycone fraction) was evaporated. The residue was dissolved in 200 µl DON solution (50 ng/ml) as the internal standard and was then introduced into the HPLC system II described below. To the aqueous layer, 0.25 ml 1 *N* NaOH, 1 ml 0.2 *M* K-phosphate buffer (pH 8.0), and 5 ml $CHCl_3/CH_3OH$ (4 : 1) were added. The resultant solution was stirred and centrifuged, then the $CHCl_3$ layer (glycoside fraction) was collected and evaporated. The residue was dissolved in 200µl AON solution (100 ng/ml) as the internal standard and then introduced into HPLC system I.

HPLC was carried out under the following conditions. HPLC system I (glycoside fraction): YMC A-312 ODS column (6 mm × 150 mm; YMC, Kyoto, Japan); mobile phase; tetrahydrofuran/1% triethylamine (adjusted to pH 2.0 with H_3PO_4) (20 : 80) excitation 485 nm, emission 550 nm; flow rate 1.0 ml/min; column temperature 40°C; injection volume 100 µ l. HPLC system II (aglycone fraction): mobile phase; CH_3CN/CH_3OH/1% triethylamine (adjusted to pH 2.0 with H_3PO_4) (35 : 15 : 50) flow rate 1.2 ml/min (other conditions the same as system I).

The ADM concentration was measured according to the following method. Plasma was diluted 1 : 1 with 1% H_3PO_4 and the mixture (1 ml) was filtered through a Sep-pack C18 column. After the column had been washed with 2 ml 0.1% H_3PO_4 and 10 ml distilled water, elution was carried out with 2.5 ml CH_3OH and the eluate was evaporated. Tissues were homogenized in 0.5 *M* KH_2PO_4/H_3PO_4 (pH 2.0) to obtain a 5% or 10% homogenate. The homogenate was diluted 1 : 1 with 80% CH_3CN containing 10 m*M* $MgCl_2$ and vigorously stirred, and the mixture was then centrifuged at 10 000 *g* for 5 min. To 1-ml of the supernatant, 2 ml saturated $NaHCO_3$ and 8 ml $CHCl_3/CH_3OH$ (4 : 1) were added and the mixture shaken for 10 min. After a 10-min centrifugation at 1100 *g*, the organic layer was collected and evaporated. The extract of plasma and tissues was dissolved in 200 µ l DM solution (200 ng/ml) as the internal standard. The mixture was centrifuged for 10 min, and the supernatant was analyzed by HPLC under the following conditions. HPLC system III: YMC A-402 phenyl column (4.6 mm × 150 mm; YMC); BPC-ph-5 guard column: (YMC); mobile phase; 25% CH_3CN/5% CH_3OH/0.5% 1 *M* $MgCl_2$ (adjusted to pH 2.0 with H_3PO_4); excitation 480 nm, emission 560 nm; flow rate 1.2 ml/min; column temperature 40°C; injection volume 100 µl.

The chromatographic system consisted of a Jasco (Tokyo, Japan) 880-PU pump, a Jasco 855-AS intelligent sampler equipped with a 100-µl sample loop, a Jasco 860-CO column oven, a Hitachi (Tokyo, Japan) F-1000 fluorescence detector and a Jasco 805-GI graphic integrator.

The within-day reproducibility of the determination of KRN8602 and its metabolites was assessed at two concentrations of 0.02 and 2 µg/g for tissues and also at two concentrations of 2 and 200 ng/ml for plasma. The coefficient of variation (CV) values for KRN8602, M1, and M2 were approximately 20% for spleen at a concentration of 0.02 µg/g, but all other CV values were 15% or lower. The rate of recovery of KRN8602 varied with each tissue and each compound, ranging from 50% to 110%, but did not vary with the drug concentration. The rate of recovery in the plasma ranged from 80% to

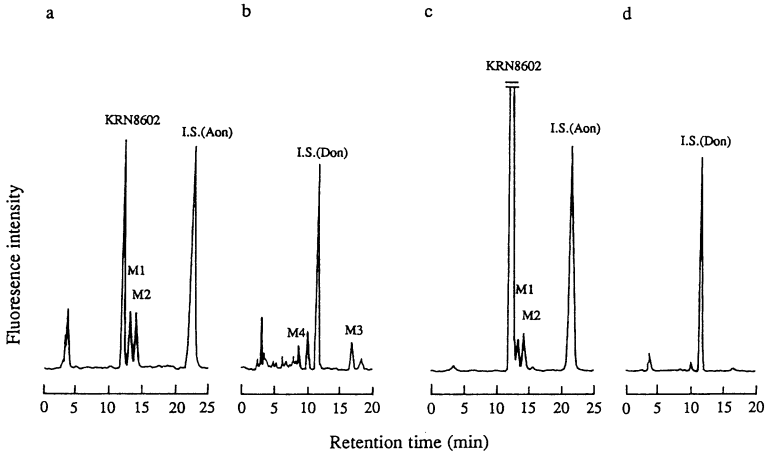

Fig. 2a–d HPLC chromatograms of plasma (**a**, **b**) and tumor (**c**, **d**) from mice 4 h after i.v. administration of KRN8602 (2 mg/kg). **a**, **c** Glycoside fraction (HPLC system I). **b**, **d** Aglycone fraction (HPLC system II)

100%, and the CV values for plasma were 10% or lower and were not dose dependent. The rate of recovery of ADM ranged from 70% to 85% in all the tissues at both concentrations (0.1 and 2 μg/g). The rate of recovery in the plasma was 90% or higher at the concentrations of 10 and 200 ng/ml, and the CV values were 8.1% or lower and were not dose dependent.

The limit of quantitation (LOQ) was determined as follows with reference to the limit of determination (LOD; Signal-to-noise ratio = 3). Briefly, the LOQ in the liver, kidney, lung, small intestine, brain, and tumor was 0.010 μg/g for KRN8602, M1 and M2, 0.005 μg/g for M3 and M4, and 0.025 μg/g for ADM. The LOQ in the heart and spleen was 0.020 μg/g for KRN8602, M1 and M2, 0.010 μg/g for M3 and M4, and 0.050 μg/g for ADM. The LOQ in the plasma was 1 ng/ml for KRN8602, M1 and M2, 0.5 ng/ml for M3 and M4, and 2 ng/ml for ADM.

Quantitative evaluation of these compounds was performed by comparing the peak height ratios of samples with those from standard curves set up in parallel with different known amounts of standards in plasma and tissues.

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated by fitting the plasma concentration–time data to a three-compartment model equation using a nonlinear least-squares program, NONLIN84 [11]. The area under the concentration–time curve (AUC) and the mean residence time (MRT) were calculated using the trapezoidal method. The clearance (CL) was calculated using the equation: CL = dose/AUC

MTT assay

An MTT assay was performed according to the method of Mossmann [12] with some modifications. In brief, drugs dissolved in RPMI-1640 medium containing 10% fetal calf serum, 50 m*M* 2-mercaptoethanol, 100 U/ml penicillin and 0.1 mg/ml streptomycin (RPMI medium) were placed in 96-well microplates at a volume of 100 μl/well at various concentrations, and 100 μl of a suspension of colon26 cells (2×10^5 cells/ml), also in RPMI medium, was added to each well. The plate was then incubated in a CO_2 incubator (5% CO_2) for 48 h at 37°C and was then centrifuged at 1100 *g* for 10 min. The supernatant was carefully removed by aspiration and 100 μl RPMI medium was added to each well. MTT [3-(4,5-dimethlthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] solution (20 μl, 5 mg/ml in PBS) was then added and the mixture was shaken for 1 min and incubated at 37°C for 4 h. After a 10-min centrifugation at 1100 *g*, the supernatant was carefully removed by aspiration, 100 μl dimethyl sulfoxide was added to each well, and the plate was shaken for 15 min to dissolve the resultant formazan. The optical density (OD 570–620 nm) was measured using a microplate reader (Model MTP-32, Corona Denki Co.). The 50% inhibitory concentration (IC_{50}) was calculated by the least squares method from the relationship between the drug concentration and the survival rate which was determined at each drug concentration with the survival rate for controls (cells only) taken as 100%. In order to evaluate the susceptibility of cells of various kinds to these drugs, a similar experiment was conducted with P388 leukemia cells.

Results

Metabolism

In mice treated with KRN8602, several metabolites as well as the parent drug were found in the plasma as shown in Fig. 2a,b. Of these metabolites, four showed the same retention times as metabolites M1, M2, M3 and M4 (Fig. 3) which had been identified in rats; they were therefore taken to be the same metabolites. In tumor tissue, some M1 and M2 was found but no M3 or M4 was detected (Fig. 2c,d). In the case of ADM, several unidentified metabolite peaks were found in the liver but the peak levels were much lower than that of the parent drug (Fig. 4c). Weak metabolite peaks were detected in plasma early on, but they were no longer detectable 1 h after administration (Fig. 4a). In tumor tissue, only parent drug was detected (Fig. 4b).

Plasma concentrations

The plasma concentrations of KRN8602 and ADM in tumor-bearing mice after i.v. administration are shown in Fig. 5, and the pharmacokinetic parameters obtained by computer analysis are summarized in Table 1.

The plasma concentrations of the two drugs after i.v. administration showed a triphasic decline with the

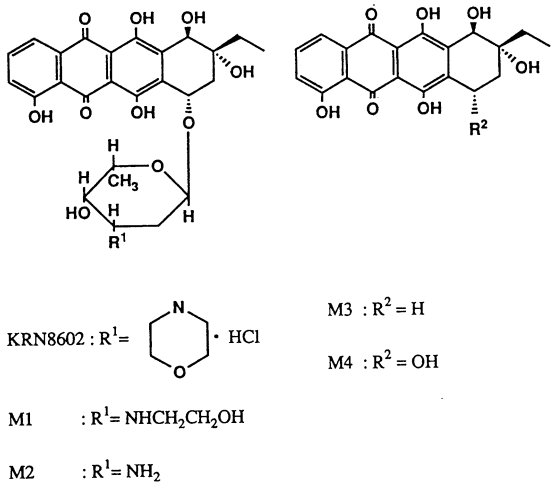

Fig. 3 Structures of KRN8602 and its metabolites

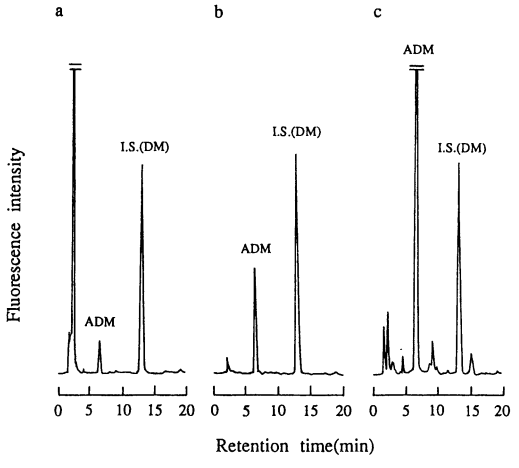

Fig. 4 HPLC chromatograms of plasma (**a**), tumor (**b**) and liver (**c**) from mice 4 h afer i.v administration of ADM (2 mg/kg)

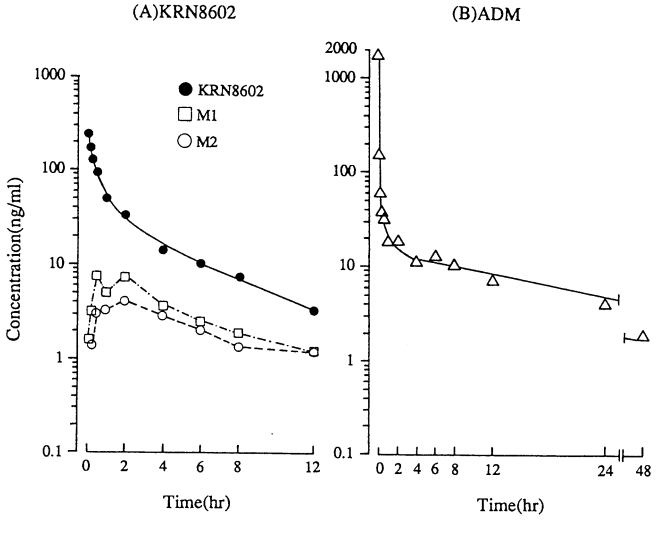

Fig. 5A, B Plasma levels of KRN8602 (**A**) and ADM (**B**) in female CDF1 mice bearing colon26 after i.v. administration of KRN8602 or ADM. Each plot show the means from three animals

Table 1 Plasma pharmacokinetic parameters of KRN8602 and ADM in female CDF1 mice bearing colon26

Parameter		KRN8602	ADM
$T_{1/2}$ (α)	(h)	0.098	0.012
$T_{1/2}$ (β)	(h)	0.660	0.334
$T_{1/2}$ (γ)	(h)	3.92	15.3
V_1	(l/kg)	5.74	0.186
V_2	(l/kg)	5.58	0.487
V_3	(l/kg)	14.7	49.1
$V_{d,ss}$	(l/kg)	26.1	49.7
Cl	(l/h/kg)	7.14	4.29
$AUC_{0\rightarrow\infty}$	(ng·h/ml)	280	466

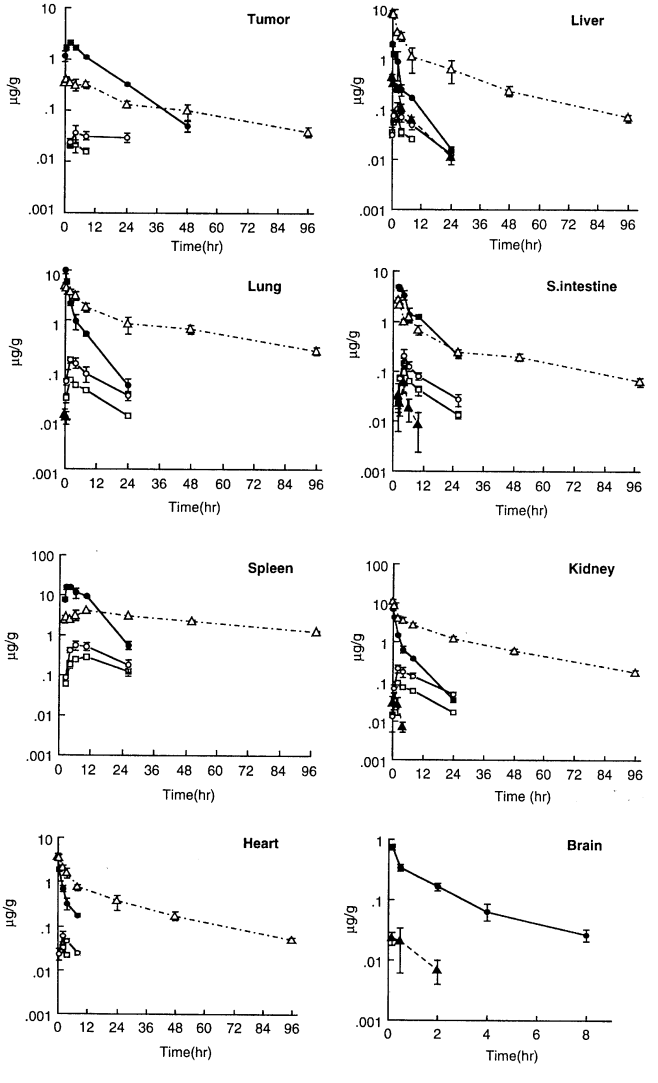

Fig. 6 Tissue levels of KRN8602 and its metabolites and ADM in female CDF1 mice bearing colon26 after i.v. administration of KRN8602 or ADM (2 mg/kg). Each plot shows the means ± SD from three animals (△ ADM, ● KRN8602, □ M1, ○ M2, ▲ M3)

following half-lives: $t_{1/2}$ (α) = 0.098 h, $t_{1/2}$ (β) = 0.660 h, and $t_{1/2}$ (γ) = 3.92 h for KRN8602; and $t_{1/2}$ (α) = 0.012 h, $t_{1/2}$ (β) = 0.334 h and $t_{1/2}$ (γ) = 15.3 h for ADM. The volume of distribution of the central compartment

Table 2 Pharmacokinetic parameters of KRN8602 and ADM in tissues of CDF1 mice bearing colon26 (*ND* not detectable, *NR* not reliable ($AUC_{finite}/AUC_{infinite}$) < 0.6)

	Drug	Peak concentration (μg/g)	$AUC_{0\to\infty}$ (μg·hr/g)	%Met[a] (%)	AUC ratio[b]	MRT (h)
Tumor	KRN8602	2.031 ± 0.225	28.92		1.99	11.63
	M1	0.021 ± 0.006	NR			
	M2	0.036 ± 0.013	NR			
	M3	ND				
	ADM	0.388 ± 0.056	14.53			48.62
Liver	KRN8602	2.009 ± 0.204	5.84		0.10	4.58
	M1	0.073 ± 0.016	NR			
	M2	0.099 ± 0.035	1.21	20.8		10.53
	M3	0.425 ± 0.065	1.99	34.1		6.38
	ADM	8.327 ± 1.127	59.85			21.52
Kidney	KRN8602	6.696 ± 0.491	14.68		0.13	4.07
	M1	0.090 ± 0.017	1.34	9.1		13.29
	M2	0.213 ± 0.048	3.41	23.2		14.38
	M3	0.029 ± 0.013	0.096	0.7		2.00
	ADM	11.076 ± 1.557	112.37			27.79
Heart	KRN8602	3.601 ± 0.358	6.27		0.17	4.27
	M1	0.031 ± 0.007	NR			
	M2	0.058 ± 0.015	0.46	7.3		7.24
	M3	0.056 ± 0.026	0.072	1.1		2.09
	ADM	3.718 ± 0.549	36.66			25.30
Spleen	KRN8602	11.321 ± 1.516	130.65		0.52	6.44
	M1	0.215 ± 0.022	5.65	4.3		21.55
	M2	0.403 ± 0.108	8.68	6.7		16.05
	M3	ND				
	ADM	2.995 ± 0.167	249.96			84.36
Lung	KRN8602	9.785 ± 1.626	20.39		0.21	4.09
	M1	0.064 ± 0.008	0.94	4.6		9.62
	M2	0.158 ± 0.021	2.30	11.3		10.53
	M3	0.013 ± 0.003	NR			
	ADM	4.878 ± 0.555	98.64			45.08
Brain	KRN8602	0.735 ± 0.078	1.14			2.97
	M1	ND				
	M2	ND				
	M3	0.023 ± 0.006	0.039	3.5		1.40
	ADM	ND				
Small intestine	KRN8602	3.906 ± 0.297	24.67		0.97	7.44
	M1	0.119 ± 0.030	1.00	4.1		11.82
	M2	0.165 ± 0.049	1.80	7.3		13.36
	M3	0.048 ± 0.018	0.20	0.8		4.70
	ADM	2.141 ± 0.258	25.37			37.41

[a] $\%Met = (AUC_{metabolite}/AUC_{KRN8602}) \times 100$
[b] $AUC\ ratio = AUC_{KRN8602}/AUC_{ADM}$

(V_1) was larger for KRN8602 (5.74 l/kg) than for ADM (0.186 l/kg), whereas the volume of distribution of the peripheral compartment (V_3) was larger for ADM.

Tissue concentrations

The changes in tissue concentrations after i.v. administration of KRN8602 and ADM to colon26-bearing mice are shown in Fig. 6. Except in the spleen and tumor, the tissue levels of both drugs reached a maximum concentration immediately after administration. In tumor, the peak concentration of KRN8602 (2.031 μg/g) was five times higher than that of ADM (0.388 μg/g). The drug concentration decreased in all tissues with time thereafter and disappearance was more rapid for KRN8602 than for ADM.

Table 2 summarizes the parameters obtained from analysis of the data on tissue concentration changes. The relative order of the AUC for KRN8602 was spleen > tumor > small intestine > lung > kidney > heart > liver > brain. For ADM, on the other hand, the relative order was spleen > kidney > lung > liver > heart > small intestine > tumor. ADM was not detected in the brain. KRN8602 showed an AUC which was twice that of ADM for tumor tissue but both were similar for the small intestine. In other tissues, the AUC ratio (KRN8602/ADM) was low at about 0.1 to 0.5. Several metabolites were found in tissues after administration of KRN8602 as described above. In tumor, the

Table 3 Apparent tissue-to-plasma concentration ratio of ADM and KRN8602 after i.v. administration. Numbers in parentheses are the ratios of the ADM value to the KRN8602 value

Tissue	Kp_{app}[a] ADM	KRN8602
Tumor	29.5 (0.20)	151.0
Liver	105.3 (4.60)	22.9
Kidney	295.2 (5.69)	51.9
Heart	70.7 (2.97)	23.8
Lung	165.7 (2.33)	71.2
Spleen	282.6 (0.30)	940.0
Small intestine	50.7 (0.38)	133.0
Brain	–[b]	3.6

[a] Kp_{app} = (ng of drug/g tissue)/(ng of drug/ml plasma)
[b] The value could not be calculated because the ADM was not detectable in the brain

peak concentrations of M1 and M2 were 0.021 and 0.036 μg/g, respectively. M3 was not detected in tumor. The peak concentrations of M1 and M2 were highest in the spleen (0.215 and 0.403 μg/g, respectively) but the AUC ratio of these metabolites to unchanged drug (%Met) was less than 7%. The peak concentration of M3 was highest in the liver (0.425 μg/g) and the%Met was 34%. The MRT of M1 and M2 tended to be longer than that of the unchanged drug. For ADM, peaks corresponding to metabolites were noted on the chromatogram, particularly for the liver; however their levels were low and the unchanged drug accounted for a large part of the total.

Table 3 shows the apparent tissue-to-plasma concentration ratio (Kp_{app}) of KRN8602 and ADM at 8 h after i.v. administration. The Kp_{app} of KRN8602 was greater than that of ADM in the tumor, spleen, small intestine and brain but it was smaller in the liver, kidney, heart and lung.

Cytotoxic activity

Table 4 shows the in vitro sensitivities of colon26 and P388 cells to KRN8602, its metabolites and ADM detected using the MTT assay. The IC_{50} value for colon26 cells was 0.13 μ*M* for KRN8602 and 2.22 μ*M* for ADM, indicating that KRN8602 is 17 times more potent than ADM. Furthermore, M1 and M2, metabolites of the glycoside type, were also more active than ADM with IC_{50} values of 1.42 and 0.15 μ*M*, respectively. M2 was similar in activity to unchanged KRN8602. In contrast, M3 and M4 were much less active than the unchanged form. The cytotoxic activities of these compounds against P388 leukemia cells were determined in a similar manner; M2 was the most potent (8.6 times the activity of KRN8602) and KRN8602 and M1 were equal in activity to ADM.

Table 4 Antitumor activity of ADM, KRN8602 and metabolites against colon26 adenocarcinoma or P388 leukemia

Drug	IC_{50} (mean ± SD, $n = 4$) colon26 (μ*M*)	P388 (n*M*)
ADM	2.22 ± 0.55	17.2 ± 2.6
KRN8602	0.13 ± 0.06	13.6 ± 2.5
M1	1.42 ± 0.21	17.2 ± 2.6
M2	0.15 ± 0.02	2.0 ± 0.5
M3	15.7 ± 3.04	5453 ± 1574
M4	9.67 ± 2.50	1856 ± 312

Discussion

In order to determine the relationship between the antitumor effect and pharmacokinetics of KRN8602, its plasma and tissue concentrations, together with those of ADM, were measured in mice bearing colon26 adenocarcinoma. In addition, the in vitro cytotoxic effects of these compounds were determined using the MTT assay. The results indicated that KRN8602 differed from ADM in its pharmacokinetic profile. In particular, KRN8602 was found to be more selective for tumors than ADM and to have much greater cytotoxic activity.

After administration of KRN8602, the unchanged form and several metabolites were detected in the plasma and tissues. Of these metabolites, M1 and M2 are formed by cleavage of the morpholino ring of KRN8602 and M3 and M4 are aglycone-type compounds produced by elimination of the amino sugar moiety (Fig. 3). The level of M3 was highest in the liver and the ratio of the AUC of M3 to that of unchanged KRN8602 was about 34%. In other tissues, the M3 level was extremely low and M3 was not detected in tumor or spleen. M1 and M2 were present in almost all tissues and their levels were relatively high in spleen, whereas the levels in tumor were very low (Fig. 6, Table 2). Although the detailed metabolic pathways for KRN8602 are now under investigation, it is suggested that there are common metabolites regardless of animal species although there is a difference in quantity (unpublished data). For ADM, on the other hand, no significant amounts of metabolites were formed in our study. Formelli et al. [4, 5] studied the metabolic fate of ADM in mice bearing colon38, and reported three metabolites: doxorubicinol (13-dihydro derivative of doxorubicin), doxorubicinone, and 7-deoxydoxorubicinone. While doxorubicinol accounted for 1% to 3% of the total fluorescent material in tumors and other tissues, these authors found two aglycones solely in the liver and these were not detected from 2 h after administration. In the present study, metabolite peaks

appeared on the chromatogram, particularly for the plasma, liver (Fig. 4a,c) and kidney, early in the post-treatment course; however, the levels were too low for the metabolites to be determined. The unchanged form alone was detected in tumor (Fig. 4b). The dose of ADM was 2 mg/kg in our study while a dose of 6 or 10 mg/kg was used in the study by Formelli et al. The reason why metabolite formation could not be satisfactorily detected in the present study may be due to the difference in dose level. For one reason or another, it may safely be said that ADM is less metabolizable than KRN8602.

KRN8602 and its metabolites and ADM were examined for cytotoxic activity against colon 26cells, KRN8602, M1 and M2 were more active than ADM, in particular, KRN8602 and M2. Against P388 leukemia cells, M2 showed the greatest cytotoxic activity and KRN8602 and M1 were equal to ADM (Table 4). It is of interest from the viewpoint of the mechanism of the cytotoxic action of these compounds and their intracellular kinetics that the intensity and pattern of cytotoxicity varied with the kind of cells, and further work remains to be done in this area. We have also studied the metabolic fate of M2 in colon26-bearing mice and have shown that transferability to tumors is extremely low for this metabolite (in which a primary amine is formed at the 3′-position by cleavage of the morpholino ring of KRN8602) when compared to the unchanged form. Details of the study results will be presented elsewhere.

Changes in plasma concentration after i.v. administration of KRN8602 differed from those after ADM administration (Fig. 5, Table 1). Elimination of KRN8602 was more rapid than that of ADM with elimination half-lives of 3.92 and 15.3 h for KRN8602 and ADM, respectively. Changes in tissue drug concentration after administration also differed between KRN8602 and ADM (Fig. 6, Table 2). Elimination of KRN8602 was more rapid than that of ADM regardless of tissue. Moreover, KRN8602 differed from ADM with respect to the distribution pattern: the AUC was large in the spleen and kidney and small in the tumor for ADM, but was large in the spleen and tumor and small in the heart and liver for KRN8602. Furthermore, it has been shown that while ADM does not transfer to the brain, KRN8602 passes into the brain at a level lower than into other tissues but higher than in plasma. KRN8602 is more lipophilic than ADM [8] and this may partly explain its penetration of the blood–brain barrier. As for tumor concentration, KRN8602 showed 5.2 times the peak concentration of ADM and two times the AUC of ADM. For all but brain tissue, in contrast, the AUC was larger for ADM than for KRN8602. Briefly, KRN8602 showed 0.098 to 0.98 times the AUC of ADM. Moreover, the Kp_{app} value of KRN8602 was 5.2 times that of ADM and it has been shown that transfer of KRN8602 into tumor tissue is marked (Table 3).

Terasaki et al. [15, 16] have reported that ADM is selectively present in cellular nuclei and that the tissue distribution of ADM is closely correlated with the DNA content of the tissue. In the present study, the Kp_{app} value was almost the same as that reported by Terasaki et al. If the tissue distribution of anthracyclines is controlled solely by the DNA content of the tissues, the distribution of the two drugs should show a similar pattern. However, the distribution pattern of KRN8602 was different from that of ADM as shown by the finding that the Kp_{app} value for KRN8602 was high in tumor and small intestine. This may suggest that not only is the DNA content of tissue important but also there are other determinants of the tissue distribution of KRN8602. This suggestion is also interesting in relation to the mechanism of action. Thus, it has been reported that the IC_{50} value is similar for ADM and KRN8602 although the amount of ADM bound to P388 cell DNA in vitro is about ten times higher than that of KRN8602 [9]. The binding of a drug to intracellular components and damage to the cell membrane may be factors in anthracycline-induced cell death. It seems unlikely, however, that the cytotoxic actions of such drugs can be completely explained by a simple mechanism of action. In the case of KRN8602, it may be appropriate to assume that a target component other than DNA is present and that the development of the action of KRN8602, mediated by the target component, is more dominant than the development of the action of ADM. The target component, however, is unknown. Clarification of this point may be of importance in increasing the selective toxicity of antitumor drugs. Intracellular distribution and kinetics will be the subjects of further studies intended to clarify the difference between KRN8602 and ADM.

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