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Pharmacokinetics and disposition of 4'-O-tetrahydropyranyladriamycin in mice by HPLC analysis

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Summary. *The plasma level of 4'-O-tetrahydropyranyladriamycin (THP) declined rapidly after IV injection to mice, with a $t_{1/2}(\alpha)$ of 0.453 min. Only 1.2 μg/ml THP was detected 2 min after injection of 5 mg/kg. The drug was immediately transferred to various tissues, where the drug levels were much higher than the plasma concentration. In the lung and spleen, 8.26 and 13.6 μg/g THP was present, respectively, 2 h after administration. Major metabolites of THP were 13-dihydro-THP, ADM, 7-deoxyadriamycinone, and 7-deoxy-13-dihydro-adriamycinone. Only 1.12% of the dose had been recovered in the urine by 48 h after injection as THP and its metabolites, according to analysis by HPLC fluorospectroscopy. The observed disposition of THP was compared with that of adriamycin (ADM). The plasma disappearance and tissue transfer of THP were faster than those of ADM. THP levels in the spleen and lung were higher and those in the heart and liver were lower than the corresponding ADM levels. Drug levels declined more quickly in most tissues in the case of THP than of ADM. Tissue distributions after single bolus and multiple injections were also compared and discussed.*

Introduction

A variety of new anthracyclines have been studied in recent years for the purpose of acquiring a better chemotherapeutic agent than adriamycin (ADM), which is limited in its clinical use because of its high cardiotoxicity, despite its high antitumor activity [5].

4'-O-Tetrahydropyranyladriamycin (THP) is a novel derivative of ADM, differing from ADM in its chemical structure in that a tetrahydropyranyl group is substituted for a hydrogen atom of the hydroxyl group at the 4' position of the aminosugar daunosamine (Fig. 1) [23]. It has been reported that THP has a higher cytocidal activity than ADM in murine leukemic cells [13] and has a potent antitumor activity against several experimental tumors [21, 22]. Cardiotoxicity studies in hamsters and rats have shown that THP is less cardiotoxic than ADM [2, 7], and clinical studies on this new anthracycline are in progress.

In this paper we present the disposition of THP in mice and compare it with that of ADM on the basis of analysis of these drugs and their metabolites by high-performance liquid chromatography (HPLC).

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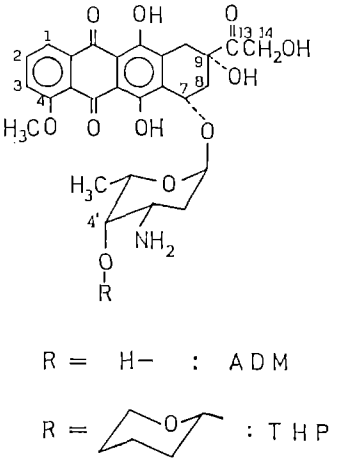

Fig. 1. Structures of 4'-O-tetrahydropyranyladriamycin and adriamycin

Materials and methods

Drugs

THP was supplied as the hydrochloride by Sanraku-Ocean Co., Ltd. ADM was obtained as the hydrochloride from Kyowa Hakko Kogyo Co., Ltd. Daunomycin (DM) was obtained from Meiji Seika Kaisha, Ltd. 13-Dihydro-THP (THP-OH), adriamycinol (ADM-OH), 7-deoxy-13-dihydroadriamycinone (7H-ADn-OH), 7-deoxyadriamycinone (7H-ADn), and 4-O-demethyl-7-deoxy-13-dihydroadriamycinone (4-OH-7H-ADn-OH) were prepared in our own laboratory. All the reference samples for HPLC analysis were identified by IR, UV, NMR, and GC-MS. THP or ADM was administered dissolved in a 0.85% NaCl solution. Because of its slow solubilization in the saline, it was first dissolved in nine-tenths of the final volume of sterilized distilled water. One-tenth of the final volume of 8.5% NaCl solution was added to give the final drug solution.

Animals, administration of drugs, and sample collection

Male ddY mice (Shizuoka Agricultural Cooperative Association for Laboratory Animals) weighing 19–21 g and 5 aged weeks received injections of 0.1 ml THP or ADM solution at a dose of 5 mg/kg into the caudal vein. The dose was chosen to give almost no toxicity [17, 19] and to be close to the effective dose determined in experimental tu-

mors in mice [22]. Each experimental group consisted of five animals, which were housed in a metabolic chamber during the experiment.

Blood samples were obtained by heart puncture 0.3, 0.6, 1, 1.5, 2, 5, 15, 30 min and 1, 2, 4, 8, 24, and 48 h after injection. The plasma was separated from blood cells by centrifugation at 2500 *g* for 10 min.

Tissues and urine were taken immediately after sacrifice of the animals 2, 8, 24, and 48 h after injection of the drug. The tissues were excised, blotted dry, and weighed for each group. The urine in the bladder was collected from all five mice in each grouop, pooled, and combined with that in the metabolic chamber, and the volume was measured.

For the tissue distribution in tumor-bearing animals sarcoma 180 tumor cells (5×10^6 cells/mouse) were inoculated to male ICR mice (5 weeks of age) SC at the groin. THP or ADM (0.45 mg/ml) was administered into the caudal vein either on a single occasion (1.5 mg/kg) or daily for 12 days (1.5 mg/kg/day × 12) from 10 days after the inoculation. The mice were sacrificed 2 or 8 h after the final injection. Tumor and tissues were immediately excised and treated as described above.

The collected plasma, blood cells, tissues, and urine were stored at −20 °C until used.

Extraction and determination of drugs

Blood. Plasma or blood cells (1 ml) burst by mixing with 2 ml water were mixed with 3 ml 0.1 *M* NH_4Cl-NH_4OH buffer (pH 9). The pH was adjusted to 9.0 with 0.5 *N*-NaOH solution if necessary. The mixture was shaken with 12 ml chloroform: methanol (2:1) for extraction. After centrifugation at 3000 *g* for 10 min the chloroform layer was collected. To the aqueous layer was added 8 ml chloroform for the second extraction. Both the first and second chloroform layers were combined and evaporated. The residue was dissolved in 2 ml chloroform: methanol (2:1) and diluted with the same solvent, if necessary, to obtain complete solubilization. To the solution was added 1 ml methanol solution of DM, which was used for an internal standard of HPLC analysis. After evaporation of the mixture containing DM the residue was dissolved in 0.2–1.0 ml acetonitrile: water (35:65). A 50- to 100-µl portion of the supernatant obtained by centrifugation at 2500 *g* for 5 min was injected into a column of HPLC.

Tissue. To 1 g tissue was added 5 ml cold 0.1 *M* NH_4Cl-NH_4OH buffer (pH 9). The mixture was treated with a homogenizer (Polytron PT-10 Kinematica) for 2–3 min at 4 °C. The homogenate was mixed with 18 ml chloroform: methanol (2:1) and shaken for 2 min after adjustment of the pH to 9 with 0.5 *N*-NaOH solution. After centrifugation at 3000 *g* for 10 min the aqueous layer was subjected to the second extraction and the chloroform layer was further processed as mentioned above for the blood sample.

Urine and bile. To 1 ml urine or bile was added 5 ml 0.1 *M* NH_4Cl-NH_4OH buffer (pH 9). The pH was adjusted to 9 with diluted NaOH solution if necessary. The mixture was shaken with 18 ml chloroform: methanol (1:1), followed by shaking for extraction. After centrifugation at 3000 *g* for 10 min the chloroform and aqueous layers were subjected to the same treatment as mentioned for the blood sample.

HPLC analysis. The determination of THP, ADM, and the metabolites of each has been described precisely in the previous paper [14]. For the detection and quantitation of these compounds a column of µ-Bondapak phenyl (Waters Assoc. USA) and a fluorescence monitor (excitation 470 nm, emission 550 nm) were used with DM as the internal standard. The mobile phase consisted of acetonitrile: 0.035 *M* HCOOH-$HCOONH_4$ buffer, pH 3 (35:65). The

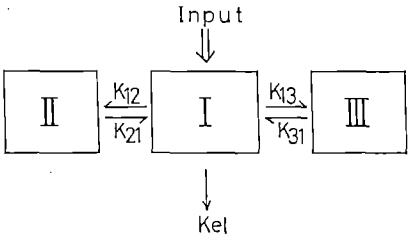

Fig. 2. Three-compartment open model. *I*, Central compartment; *II*, rapidly equilibrated peripheral compartment; *III*, slowly equilibrated peripheral compartment; K_{ij}, apparent first-order rate constant for transfer of drug from the i-th to the j-th compartment; K_{el}, elimination rate constant of the central compartment

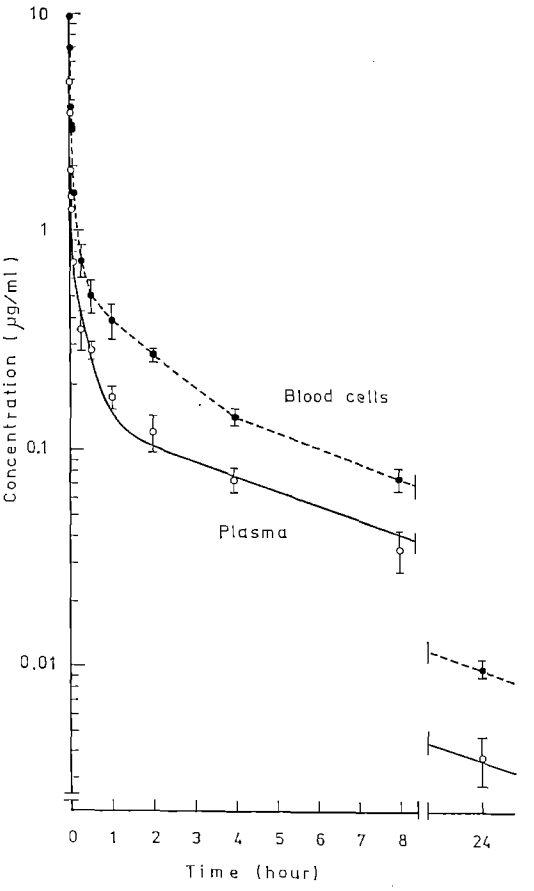

Fig. 3. Plasma and blood cells levels of THP in mice. THP was administered to mice at a dose of 5 mg/kg IV. *Bars* represent standard errors for the means of values from five mice. Plasma levels were simulated by a three-compartment open model as shown in Fig. 2.

retention times of ADM-OH, 7H-ADn-OH, ADM, 7H-ADn, 4-OH-7H-ADn-OH, THP-OH, DM, and THP were 4.8, 5.5, 6.8, 8.8, 9.0, 9.5, 11.5, and 16.2 min, respectively. 4-OH-7H-ADn-OH was calculated as 7H-ADn because the retention times of these two compounds mostly overlapped with each other.

The recoveries of THP added to the plasma and blood cells were 95%–98% according to this analysis. Those of ADM added to the plasma and blood cells were 90%–95% and 85%–90%, respectively. The lower limit of detection for THP in this analysis was 0.5 ng injection sample (1.25 ng/ml or ng/g of original sample) with the 'spiked' sample as shown in [14].

Pharmacokinetic analysis

Pharmacokinetic analysis of plasma disappearance of THP and ADM was performed according to a three-compartment open model. This model consists of two peripheral compartments and a central compartment containing an exit route, as shown in Fig. 2. The 'NONLIN '74' computer program [16] was used to determine the values of parameters by nonlinear regression analysis from the plasma concentration–time data. The data were fitted to the following equation:

$$C = Ae^{-pt} + BE^{qt} + De^{-rt}$$

where C : plasma concentration of drug.

Table 1. Pharmacokinetic parameters of THP and ADM in mice

		THP	ADM
A	(ng/ml)	6824.0	41726.0
B	(ng/ml)	638.0	188.0
D	(ng/ml)	140.0	40.8
p	(h^{-1})	91.5	54.1
q	(h^{-1})	3.05	1.99
r	(h^{-1})	0.153	0.0402
$t_{1/2}(\alpha)$	(h)	0.0076	0.0128
$t_{1/2}(\beta)$	(h)	0.227	0.348
$t_{1/2}(\gamma)$	(h)	4.53	17.24
K_{12}	(h^{-1})	61.28	5.69
K_{21}	(h^{-1})	11.73	2.23
K_{13}	(h^{-1})	14.78	25.82
K_{31}	(h^{-1})	0.573	0.0870
K_{el}	(h^{-1})	6.34	22.31
V_1	(l/kg)	0.658	0.119
V_2	(l/kg)	3.44	0.304
V_3	(l/kg)	16.95	35.35
V_d	(l/kg)	21.04	35.78
Cl	(l/h·kg)	4.17	2.65
AUC	(ng·h/ml)	1199.0	1881.0

Plasma levels were simulated by a three-compartment open model as shown in Fig. 2 and the following equation:

$C = Ae^{-pt} + Be^{-qt} + De^{-rt}$

$t_{1/2}$, Half-life of the drug; K_{ij}, apparent first-order rate constant for transfer of drug from the i-th to the j-th compartment; K_{el}, elimination rate constant of the drug from the central compartment; V_i, apparent volume of distribution in the i-th compartment; V_d, apparent volume of distribution at steady state; Cl, plasma clearance; AUC, area under the plasma concentration-time curve

Pharmacokinetic parameters were calculated as follows:

$t_{1/2}$: Half-life of drug
$t_{1/2}(\alpha) = 0.693/p$
$t_{1/2}(\beta) = 0.693/q$
$t_{1/2}(\gamma) = 0.693/r$

V_i: Apparent volume of distribution in the i-th compartment

X_0: Dose

V_d: Apparent volume of distribution at steady state
$V_d = V_1 + V_2 + V_3$
$V_1 = X_0/(A+B+D)$
$V_2 = V_1 \cdot K_{12}/K_{21}$
$V_3 = V_1 \cdot K_{13}/_{31}$

Cl: Plasma clearance $Cl = V_1 \cdot Kel$

AUC: Area under the plasma concentration–time curve
$AUC = (A+B+D)/Kel$

Results

Blood levels of THP in mice given THP

Plasma and blood cell concentrations of THP in mice after IV injection are shown in Fig. 3. THP disappeared very

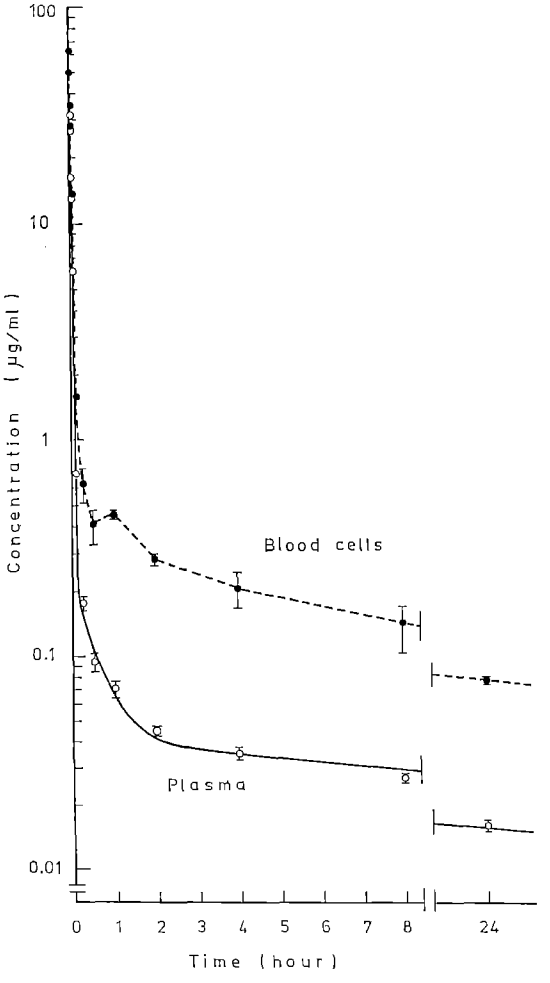

Fig. 4. Plasma and blood cells levels of ADM in mice. ADM was administered to mice at a dose of 5 mg/kg IV. *Bars* represent standard errors around the means of values from five mice. Plasma levels were simulated by the three-compartment open model as shown in Fig. 2

Table 2. Tissue levels of THP and the metabolites in mice

	Time (h)	Glycosides					Aglycones			Total
		THP	THP-OH	ADM	ADM-OH	Total	7H-ADn-OH	7H-ADn	Total	
Kidney	2	6.81	–	0.67	–	0.67	–	–	–	7.48
	8	1.74	–	0.68	0.12	0.80	0.07	0.74	0.81	3.35
	24	0.21	–	0.20	0.09	0.29	0.10	0.46	0.56	1.06
	48	0.03	–	0.11	–	0.11	0.02	0.14	0.16	0.30
Liver	2	4.70	–	2.96	–	2.96	2.39	1.07	3.46	11.1
	8	1.74	0.05	2.54	0.52	3.06	0.86	1.42	2.28	7.08
	24	0.47	–	0.26	–	0.26	1.20	0.85	2.05	2.78
	48	0.18	–	0.16	–	0.16	tr	0.11	0.11	0.45
Lung	2	8.26	–	tr	–	tr	–	–	–	8.26
	8	216	–	0.51	–	0.51	0.07	0.56	0.63	3.30
	24	0.19	–	0.14	–	0.14	0.02	0.31	0.33	0.66
	48	0.03	–	0.05	–	0.05	–	–	–	0.08
Heart	2	2.04	–	0.31	–	0.31	0.16	0.15	0.31	2.66
	8	0.35	–	0.28	0.05	0.33	0.06	0.32	0.38	1.06
	24	0.04	–	0.09	–	0.09	0.03	0.14	0.17	0.30
	48	–	–	0.08	–	0.08	–	0.10	0.10	0.18
Spleen	2	13.9	–	–	–	–	–	–	–	13.9
	8	8.52	–	0.90	–	0.90	–	0.61	0.61	10.0
	24	1.25	–	0.44	–	0.44	–	0.42	0.42	2.11
	48	0.20	–	0.19	–	0.19	–	0.14	0.14	0.53
Thymus	2	1.92	–	0.08	–	0.08	0.04	tr	0.04	2.04
	8	2.26	–	0.08	–	0.08	0.03	tr	0.03	2.37
	24	2.14	–	0.13	–	0.13	–	0.14	0.14	2.41
	48	1.03	–	0.16	–	0.16	–	tr	tr	1.19
Bile	2	18.6	–	9.71	–	9.71	–	–	–	28.3
	8	10.7	–	12.6	0.98	13.6	1.58	tr	1.58	25.9
	24	–	–	3.10	–	3.10	–	–	–	3.1
	48	–	–	0.41	–	0.41	–	–	–	0.41
Blood cells	2	0.18	0.007	0.018	–	0.025	0.013	0.007	0.020	0.225
	8	0.046	0.003	0.012	–	0.015	0.009	0.008	0.017	0.078
	24	0.007	–	0.006	–	0.006	0.003	0.006	0.009	0.022
	48	–	–	–	–	–	–	–	–	–
Plasma	2	0.12	–	0.008	–	0.008	0.011	0.008	0.019	0.147
	8	0.032	–	0.004	–	0.004	0.006	0.005	0.011	0.047
	24	0.004	–	tr	–	tr	0.002	0.002	0.004	0.008
	48	–	–	–	–	–	–	–	–	–

tr, trace; –, not detected

The values are the mean concentrations (μg/g) of five mice. The concentrations of metabolites are expressed as THP equivalents. Mice received 5 mg/kg THP IV and were sacrificed 2, 8, 24, or 48 h after drug injection. THP and the metabolites were extracted from the tissues and analyzed by HPLC as described in *Materials and methods*

rapidly from the plasma after IV administration. Even 0.3 min after injection the plasma level was 4.9 μg/ml, which was not high considering that the dose was 5 mg/kg. The value fell further to 1.2 μg/ml 2 min after injection. The blood level of THP was higher in the blood cells than in the plasma through out the observation period in this experiment (0.3 min to 24 h after injection). The blood cell level was about twice as high as the plasma level from 30 min to 24 h after administration.

The plasma level of THP fitted well to the concentration–time curve simulated by computer analysis assuming a three-compartment open model. The parameters calculated by pharmacokinetic analysis of the data on THP obtained up to 24 h after injection are shown in Table 1. The half-lives ($t_{1/2}$) in the alpha, beta, and gamma phases were 0.0076, 0.23, and 4.53 h, respectively.

Blood levels of ADM in mice given ADM and comparison of the two drugs

The blood levels, in the form of both plasma and blood cell concentrations, of ADM are given in Fig. 4. The plasma level of ADM was 30.9 μg/ml 0.3 min after IV injection, which was higher than that of THP. Rapid disappearance of ADM from the plasma was also observed. The blood cell concentration of ADM was higher than the plasma level, as in the case of THP. It was about 4–6 times higher than the plasma levels, or twice as high in the case of THP, between 30 min and 24 h after administration of drug.

The pharmacokinetic parameters obtained by computer analysis are shown in Table 1. THP disappeared from plasma immediately after the injection, and more rapidly than ADM, which was seen in the plasma half-life in the

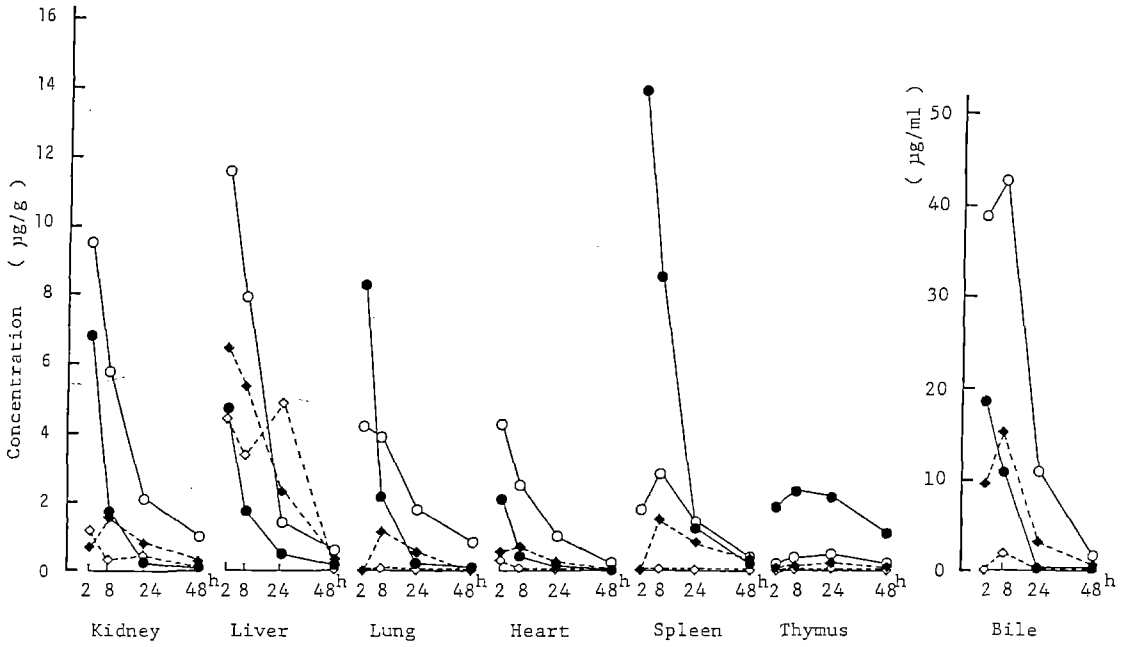

Fig. 5. Tissue levels of THP, ADM and their metabolites in mice. The *plots* represent the mean concentrations of THP (●), the metabolites of THP (◆), ADM (○), and the metabolites of ADM (◇). Mice received THP or ADM, 5 mg/kg IV and were sacrificed at 2, 8, 24, and 48 h after injection of drugs. THP, ADM, and their metabolites were extracted from tissues and analyzed by HPLC as stated in *Materials and methods*

α-phase. However, the plasma level of THP from 0.5 h to 8 h after administration was higher than that of ADM, because the α-phase of THP was shorter than that of ADM. The elimination rate was faster for THP than for ADM in the γ-phase, and the half-lives of THP and ADM were 4.53 and 17.24 h, respectively. The rapid disappearance of THP from plasma in the α-phase was reflected in the fact that the rate constant K_{12} of THP was higher than that of ADM. The apparent volume of distribution in the slowly equilibrated peripheral compartment V_3 in the mice to which THP was injected was smaller than that in the mice that received ADM. This was attributed to the lower rate constant K_{13} and the higher K_{31} of THP than of ADM.

Tissue distribution of THP and its metabolites in mice given THP

The tissue distributions of THP and its metabolites in mice treated with a dose of THP at 5 mg/kg IV are shown in Table 2 in Fig. 5. The tissue levels of THP were 13.9 μg/g in the spleen, 8.26 μg/g in the lung, and 6.81 μg/g in the kidney 2 h after administration, all of which were higher than in other tissues. THP was distributed to the liver, heart, and thymus at concentrations of 4.70, 2.04, and 1.92 μg/g, respectively. The levels in these organs were reduced to less than 1 μg/g 8 h after administration, except in the spleen and thymus. Metabolites of THP found in the organs of mice were glycosides, THP-OH, ADM, and ADM-OH, and aglycones, 7H-ADn and 7H-ADn-OH, as shown in Table 2. High levels of the metabolites were found in the liver.

Tissue distribution of ADM and its metabolites in mice given ADM and comparison of the two drugs

The distribution of ADM and its metabolites to various tissues in mice given ADM are shown in Table 3 and Fig. 5. The concentration of ADM was higher in the liver and kidney 2 and 8 h after injection of ADM. These levels of ADM were higher than those of THP in mice given THP. Also, drug concentrations in the heart were found to be higher 2 h and longer after the injections in mice given ADM than in those that received THP. THP levels declined quickly in most organs except the thymus, in which THP persisted until 48 h after injection. Although the concentration of THP or ADM in the bile 2 or 8 h after injection was higher than that in organs or urine, the level of THP was lower than that of ADM.

Major metabolites found in mice given ADM were ADM-OH and 7H-ADn-OH, as shown in Table 3. ADM-OH was detected in most tissues of the mice treated with ADM, while little or no THP-OH was detected in any tissues of the mice treated with THP. In the case of mice given THP the total level of the metabolites was higher than the level of unchanged THP 24 h after injection in bile and organs except for thymus and spleen. On the other hand, only small amounts of metabolites of ADM or none at all were found in any organs other than liver and kidney in the mice treated with ADM, and unchanged ADM was still at quite a high level 24 h after injection.

Distribution of the two drugs in the heart of mice

The concentrations of THP or ADM in the heart were observed 2, 5, 10, 15, 30 min, 1, 2, 8 and 24 h after injection of THP or ADM to the mice at a dose of 5 mg/kg. As shown in Fig. 6, the concentration of THP was higher initially than that of ADM, and showed a peak at 2 min or earlier. The THP level decreased rapidly within the first 1 h and then fell to a negligible level by 24 h after the injection of THP. In contrast, the peak ADM level in the heart in mice given ADM was 10–15 min after injection. The decline in ADM level was slow, and the concentration of ADM was about 10 times higher than that of THP 8 h after injection, which remained higher until 24 h.

Table 3. Tissue levels of ADM and the metabolites in mice

	Time (h)	ADM	Glycoside	Aglycones			Total
			ADM-OH	7H-Adn-OH	7H-ADn	Total	
Kidney	2	9.45	0.58	0.61	tr	0.61	10.64
	8	5.69	0.23	0.04	0.10	0.14	6.06
	24	2.07	0.16	0.15	0.16	0.31	2.54
	48	0.98	0.11	0.02	0.03	0.05	1.14
Liver	2	11.5	0.49	3.39	0.55	3.94	15.93
	8	7.93	0.45	2.21	0.74	2.95	11.33
	24	1.44	0.13	3.86	0.90	4.76	6.33
	48	0.60	0.09	0.02	0.01	0.03	0.72
Lung	2	4.11	–	tr	–	tr	4.11
	8	3.86	–	0.06	–	0.06	3.92
	24	1.81	0.03	0.06	0.03	0.09	1.93
	48	0.76	0.02	tr	tr	tr	0.78
Heart	2	4.24	0.14	0.19	–	0.19	4.57
	8	2.46	0.06	0.04	0.03	0.07	2.59
	24	1.04	0.05	0.03	0.04	0.07	1.16
	48	0.28	tr	–	–	–	0.28
Spleen	2	1.78	tr	0.05	tr	0.05	1.83
	8	2.79	tr	0.03	0.04	0.07	2.86
	24	1.46	–	–	0.03	0.03	1.49
	48	0.30	–	–	–	–	0.30
Thymus	2	0.22	0.01	0.05	–	0.05	0.28
	8	0.32	0.01	0.02	–	0.02	0.35
	24	0.43	0.03	–	tr	tr	0.47
	48	0.20	–	–	–	–	0.20
Bile	2	38.7	tr	tr	–	tr	38.7
	8	42.6	2.04	0.43	tr	0.43	45.07
	24	10.9	tr	–	–	–	10.9
	48	1.63	tr	–	–	–	1.63
Blood cells	2	0.141	0.011	0.024	0.005	0.029	0.181
	8	0.071	0.006	0.011	tr	0.011	0.088
	24	0.038	0.009	0.012	–	0.012	0.059
	48	0.019	0.009	0.002	–	0.002	0.030
Plasma	2	0.022	–	0.017	0.003	0.020	0.042
	8	0.013	–	0.006	tr	0.006	0.019
	24	0.008	–	0.003	tr	0.003	0.011
	48	0.003	–	–	–	–	0.003

tr, trace; –, not detected

The values given are the mean concentrations (μg/g) of five mice. The concentrations of metabolites are expressed as ADM equivalents. Mice received 5 mg/kg ADM IV and were sacrificed 2, 8, 24, or 48 h after drug injection. ADM and the metabolites were extracted from the tissues and analyzed by HPLC as described in *Materials and methods*

Urinary excretion of THP

The urinary excretion of THP in mice was monitored for 48 h after IV administration of THP at a dose of 5 mg/kg (Fig. 7). The urine excreted in the first 8 h contained THP and metabolites accounting for about 0.6% of the dose. Over 48 h after injection of drug the total recovery of THP and the metabolites was only 1.12% of the administered dose. Unchanged THP accounted for about 17% of the total recovery and the aglycone metabolites 7H-ADn, 7H-ADn-OH and 4-OH-7H-ADn-OH, about 75% in 48 h in mice treated with THP.

Urinary excretion of ADM and comparison of the two drugs

The urinary excretion of ADM in mice given ADM is also shown in Fig. 7. The cumulative excretion rate of ADM up to 8 h after administration was almost the same as that of THP. But the cumulative excretion rate of the sum of ADM and the metabolites over 48 h was about double that of THP and metabolites in mice given THP. The ratio of unchanged ADM to the total excreted compounds was 82%, which was much higher than 17% in the case of THP.

Tissue distribution of THP and ADM in sarcoma 180 solid-tumor-bearing mice after single and multiple administration

Tissue levels of THP, ADM, and their metabolites in a mutliple dose-schedule were examined 2 and 8 h after the last injection and compared with the results of a single

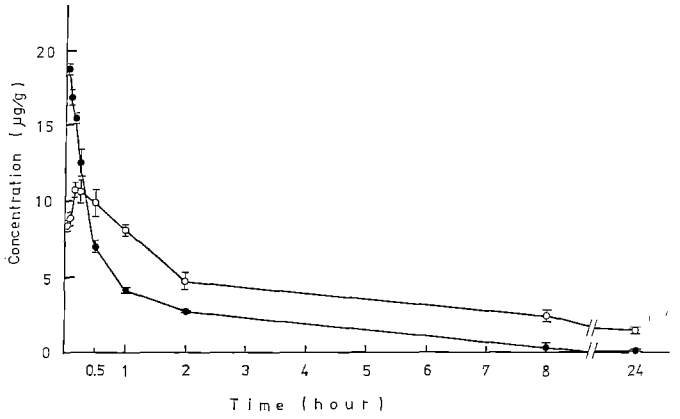

Fig. 6. Heart levels of THP and ADM in mice. The *plots* represent the mean concentrations of THP (●) and ADM (○). *Bars* represent the standard error about the means for five mice in each case. Mice received THP or ADM, 5 mg/kg IV and were sacrificed at each time interval shown after injection of drugs. The hearts were excised and analyzed by HPLC as stated in *Materials and methods*

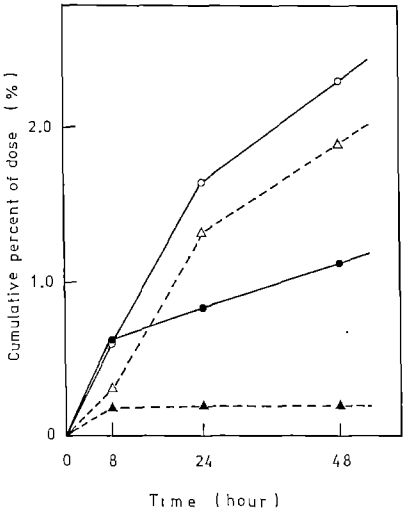

Fig. 7. Urinary excretion of THP and ADM in mice. The *plots* represent urinary excretion of unchanged THP (▲), the total (●) of THP and the metabolites, unchanged ADM (△), and the total (○) of ADM and the metabolites, expressed as the mean cumulative percentage of the dose administered to five mice. Mice were housed in metabolic cages after IV injection of THP or ADM at a dose of 5 mg/kg. Urine was collected without catheters at all times. Extraction and analysis were performed as described in *Materials and methods*

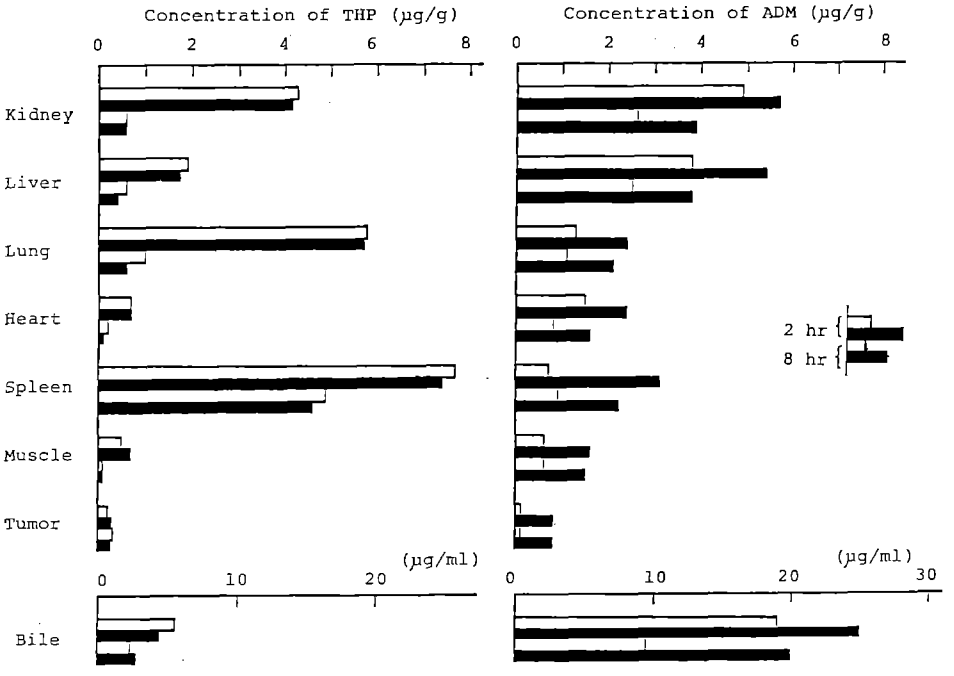

Fig. 8. Tissue levels of THP and ADM after single or multiple administration in solid-tumor-bearing mice. The histograms represent the mean concentration of unchanged THP and ADM in five mice after single (□) and multiple (■) administration. Mice received THP or ADM every day for 12 days (1.5 mg/kg/day) from 10 days after inoculation of s-180 tumor cells, and were sacrificed 2 or 8 h after the first or final administration of drugs. THP, ADM, and their metabolites were extracted from tissues and analyzed by HPLC as mentioned in *Materials and methods*

dose of THP or ADM in the mice bearing solid tumors (Fig. 8). In the group of mice treated with a single dose of 1.5 mg/kg THP, a higher level of THP was found in the spleen and lung, as in the normal mice described above. A lower level was found in the heart, muscle, and tumor.

The tissue levels of THP in mice that received mutliple treatment with THP were almost equal to those in animals in the single-dose group. On the other hand, the concentration of ADM in all the tissues investigated from mice given ADM were markedly higher in the multiple-dose group

than in the single-dose group. No accumulation of drug was found following multiple injections in mice treated with THP but it was in mice treated with ADM. Accordingly, 8 h after the last injection of the multiple treatment at the same dose of the two drugs the drug concentrations in all tissues except for spleen were considerably higher in mice given ADM than in mice given THP. For example, ADM levels were about 10 times higher than THP levels in the heart and muscle, although about 3 times higher in the lung and tumor.

Discussion

There have been many studies on the pharmacokinetics and disposition of ADM in several animal species [24] and in humans [4]. We have already studied the disposition of a new anthracycline, aclacinomycin [9–11]. In this study we revealed that THP disappeared more rapidly than ADM from the plasma in mice immediately after IV administration. Pharmacokinetic analysis of plasma levels of THP and ADM by simulation based on a three-compartment open model showed that THP injected IV was more rapidly transferred to tissues than ADM, especially to the rapidly equilibrated peripheral compartment. This result shows faster uptake of THP than ADM by mammalian cells. It has been reported that THP was taken up very rapidly by mouse leukemia cells in vitro. The rate of uptake of THP was about 170 times faster than that of ADM in the initial phase [13]. This may have a relation to the fact that THP is more lipophilic than ADM as seen in the retention time on reversed-phase HPLC [14] or the Rf value on silica gel TLC analysis.

On the tissue distribution of drug, a substantial difference was found between THP and ADM in mice. A pharmacokinetic study of ADM in mouse organs was reported by Formelli et al. [8]. THP levels found in the spleen and lung were higher and those in the heart and liver were lower than those of ADM 2 h after drug injection. However, the decline in THP levels in major organs, except for the thymus, was more pronounced than that of ADM. In many organs the level of THP metabolites was much higher than that of ADM metabolites. Consequently accumulation of the original drugs, i.e. THP or ADM, in the tissues after multiple treatment was found in mice treated with ADM but not in mice treated with THP. The accumulation of ADM in several mouse tissues has been reported by Siemann et al. [20]. and Campeneere et al. [6]. These authors have discussed a possible relationship of the drug accumulation to toxic side effects. According to Dantchev et al. [7] and our own study [12], THP has lower cardiotoxicity in hamsters and rats than ADM. These results suggests that the drug accumulation in the heart of animals correlates to the cardiotoxicity.

The concentration of THP was lower in tumor than in other tissues of mice, but seemed to attain or exceed the level needed to develop a cytocidal effect. The levels of THP and ADM that are cytocidal against L1210 cells have been reported to be about 0.003 and 0.016 μg/ml (IC_{50}) [15], respectively.

The metabolism of ADM has been studied by Yesair et al. [24] and Benjamin et al. [4], who found the major metabolite to be 13-dihydroadriamycin, adriamycinol. The metabolites formed by reduction of the ketone group at the C-13 position of ADM and THP are ADM-OH and THP-OH, respectively. The former was found in most tissues of mice injected ADM but the latter was not detected in any dissues except the liver and blood of mice that had received THP. THP seems to be less favourable than ADM for the substrate of aldo-keto reductase which catalyzes the carbonyl reduction [1].

ADM detected in the tissues of mice given THP was regarded as a metabolite of THP because ADM was found in the liver or bile in greater quantities more than other tissues and because we detected formation of little or no ADM as an artifact during extraction or analysis procedures. However, we could not obtain sufficient evidence to support in vivo formation of ADM from THP by our in vitro experiment with a tissue homogenate or blood sample. A trace of ADM formation was observed in the liver homogenate or blood of mice after incubation with THP and co-factors under aerobic or anaeobic conditions. If THP was first decomposed to ADM exclusively and then metabolized to ADM-OH and aglycones, the metabolite levels would be almost same in animals given ADM at the same dose. But in mice given THP the level of ADM-OH was lower and that of aglycones was higher than in animals given ADM (Tables 2 and 3). Therefore, some of the THP given to mice may be converted to ADM before further decomposition, but it is unreasonable to suppose that an entire THP is metabolized through formation of ADM. We consider that there is a multiple-pathway for metabolism of THP in mice.

We used an analytical method of reversed-phase HPLC in which the aglycones, adriamycinone and 13-dihydroadriamycinone, were well separated from 7H-ADn and 7H-ADn-OH. In our study the former two aglycones were detected only in very small quantities or not at all in the blood or tissues of mice treated with THP or ADM.

Many anthracyclines has been reported to be excreted in smaller amounts than water-soluble antibiotics in the urine of experimental animals or humans after IV administration, probably because anthracyclines persist in the tissues for a long time. The cumulative excretion of THP and the metabolites in the urine of mice was about half that of ADM and the metabolites over 48 h after administration at the same dose. The drug concentration in the bile was lower in mice given THP than in mice given ADM, but it was much higher than that in the urine. ADM has been reported to be excreted more in the bile than in the urine after injection of ADM in rats [24], rabbits [3], and patients [18]. The biliary excretion of THP was estimated to be less than that of ADM from the data of drug concentrations in the bile in this experiment. But it might be speculated that THP has a similar excretion pattern, to ADM, i.e., more is excreted in the bile than in the urine.

The lower excretion rate of THP and its metabolites in the bile and urine, despite their rapid disappearance from the blood and tissues compared with ADM, may indicate that THP is metabolized to an unidentified compound not detectable by the present analytical method with fluoroscopy. This should be discussed after further studies with labeled THP.

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