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Abstract

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KEYWORDS: adriamycin, doxorubicin, pharmacokinetic analysis, high-performance liquid chromatography, Ehrlich tumor

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PHARMACOKINETIC ANALYSIS OF ADRIAMYCIN (DOXORUBICIN) AND RELATED FLUORESCENT COMPOUNDS IN EHRLICH TUMOR-BEARING MOUSE PLASMA AND TISSUES

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Abstract. Pharmacokinetic analysis of the distribution and concentration of adriamycin (ADM) in mouse plasma and tissues was carried out by differentiating the unmetabolized form from metabolized ones using high-performance liquid chromatography after a single intravenous injection. Marked differences between ADM and total ADM equivalent values (total ADM values) or its metabolized forms were observed in the pharmacokinetic behavior in plasma and tissue distributions. The ratios of tissue per plasma for total ADM and for ADM values in the liver, kidney and heart showed a two-digit magnitude each time they were examined. Twenty four h later, the ratios for ADM values in the liver, kidney, heart and lung were at high levels; 43.1, 48.1, 57.9 and 45.5 times, respectively. Twenty min after injection the ratios for total ADM values in the spleen, lung and tumors were comparatively small, but 24 h later, the ratio had increased 36.5, 45.5 and 6.8 times respectively.

Key words : adriamycin, doxorubicin, pharmacokinetic analysis, high-performance liquid chromatography, Ehrlich tumor.

Adriamycin (ADM) is an anthracycline antibiotic used extensively for the treatment of leukemia and malignant tumors (1). Its initial half-life time in plasma administered by intravenous injection is very short (2-4) and it has a strong affinity for tissue proteins (5-7) and deoxyribonucleic acid (8). Arena *et al.*, (5) first reported that concentrations in most tissues in mice were of at least one or two orders of magnitude greater than those in blood. Similar results were reported thereafter (3, 6).

When ADM shows its biological behavior in plasma or tissues, it is important to note that it may exist as either active or inactive forms at a site. In varying amounts, ADM has been metabolized to aglycones or polar metabolites in biological fluids (7, 9, 10, 11). Therefore, some ADM is considered to be metabolized in most tissues.

In the present study, we examined the pharmacokinetic behavior of ADM and related fluorescent compounds in mouse plasma and tissues.

MATERIALS AND METHODS

Reagents. Adriamycin hydrochloride, adriamycinone and adriamycinol were kindly donated by Farmitalia (Milan, Italy). The chloroform, isopropanol, acetic acid and sodium acetate used were of analytical grade.

Animal experiments. Ehrlich ascites tumors (2×10^6 cells per animal) were inoculated on the backs of ICR male mice (30-35 g in body weight, 6 weeks old). The mice had free access to food and water. Seven days later, the mice were anesthetized by inhalation of ether and injected intravenously with ADM sterilized saline solution (1 mg/ml) at a dose of 2.8 mg/kg using a syringe with a Harvard Apparatus infusion pump into the right inferior vana cave (0.53 ml/min). The mice were then sacrificed at definite intervals (20 min, l, 5, 12 and 24 h after the injection) by cutting the cervical artery. The plasma was isolated. There were 3 mice in each group. The liver, kidney, spleen, lung, heart, duodenum (with contents) and tumor tissues were excised, washed with sterilized saline solution and cut into small pieces in 2 ml of 10 mM phosphate-buffered saline (pH 7.8). The pieces of the organs were homogenized with a Polytron homogenizer to make a 5-10 % homogenate. The homogenates were stored at −20° in the dark until used for measurement.

Determination of ADM and related fluorescent compounds in the plasma and tissues. The concentrations of ADM and its related fluorescent compounds in the plasma and tissues of Ehrlich tumor-bearing mice were determined by high-performance liquid chromatography (HPLC) (12). In brief, a Hitachi Model 635A high-performance liquid chromatograph was connected to a Hitachi Model 650-10S high-sensitivity fluorescence spectrophotometric detector. The results were recorded on a Hitachi 056 recorder and calculated by a Hitachi model 834-30 Chromato-Processor as an integrator using the ratio of the peak area to standard ADM solution.

HPLC was carried out using Zorbax Sil in the stationary phase, chloroform-isopropanol-acetic acid-water-sodium acetate buffer (pH 4.5) (100 : 100 : 14 : 14 : 1) in the mobile phase at a flow-rate of 1.0 ml/min with a fluorescence spectrophotometric detector at an excitation wavelength of 470 nm and an emission wavelength of 585 nm. The extraction procedure of ADM and its recovery rate from the plasma or tissues have been described previously (7). All operations with ADM and related fluorescent compounds were carried out in near darkness.

RESULTS

The P1-P7 metabolites of ADM related fluorescent compounds were detected in ADM-administered mouse plasma and tissues by the method reported above. In the previous experiment, the P2 metabolite was identified as adriamycinone and P6 as adriamycinol. The contaminating biological blank was detected at the same site as P5, but in trace amounts. The AD-NE indicates the total concentrations of P2 plus P3 metabolite.

Fig. 1 shows the concentrations of ADM and its related fluorescent compounds as detected in Ehrlich tumor-bearing mouse plasma after a single intravenous injection (2.8 mg/kg), examined by the HPLC method. The values are expressed as the mean average of 3 mice plotted on a semilog scale.

When pharmacokinetic analysis is applied to these data, it is evident that

these concentrations (C) can be adequately described by two exponential functions of time, thus,

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{.....formula 1}$$

Absorption

↓

Metabolism, Excretion ←K10— Central compartment ⇄K12 K21 Peripheral compartment

Scheme 1

$$K12 + K21 + K10 = \alpha + \beta \qquad K21 \cdot K10 = \alpha \cdot \beta \qquad K21 = \frac{A\beta + B\alpha}{A + B} \qquad V = \frac{\text{Dose}}{A + B}$$

.....formula 2

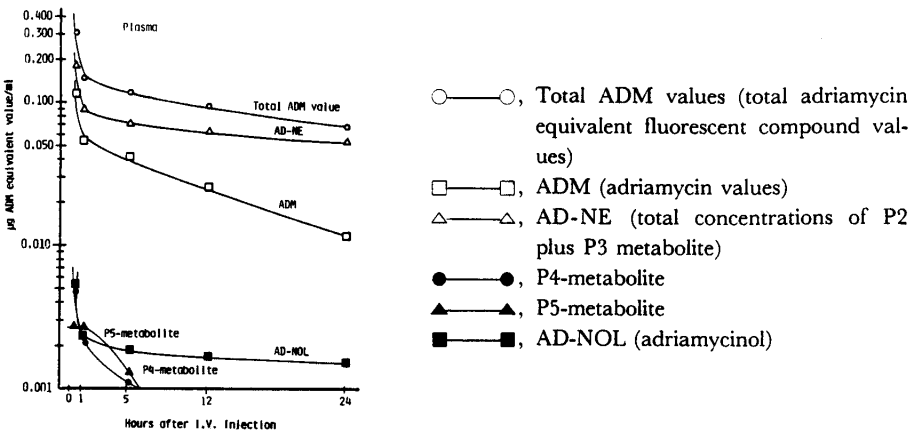

Fig. 1 Concentrations of ADM and its related fluorescent compounds in Ehrlich tumor bearing mouse plasma after a single intravenous injection (2.8 mg/kg), examined by HPLC. The values are expressed as the mean of 3 mice.

Consider now the distribution of ADM in a two-compartment open model according to Scheme 1. From the graphically (Fig. 1) obtained value of A, B, α and β, the rate constants can be readily computed (13). Then the values are inserted in formula 2. In this manner, the values of apparent volume of distribution (V), biological half-life time ($t \cdot 1/2$) and body clearance ($V \cdot K10$) were calculated.

The elimination curve of total ADM equivalent fluorescent values (total ADM values) in the plasma was similar to that of AD-NE. Its half-life time ($t \cdot 1/2$) was 26.5 h and that of AD-NE was 34.0 h. On the other hand, ADM (in unmetabolized form) showed a comparatively rapid clearance from the plasma, its

half-life time was 11.2 h. The concentration of P4-metabolite and P5-metabolite 6 h after injection was below 1.0 ng/ml (Fig. 1 and Table 1).

Figs. 2 and 3 show the behaviors of ADM and its related fluorescent com-

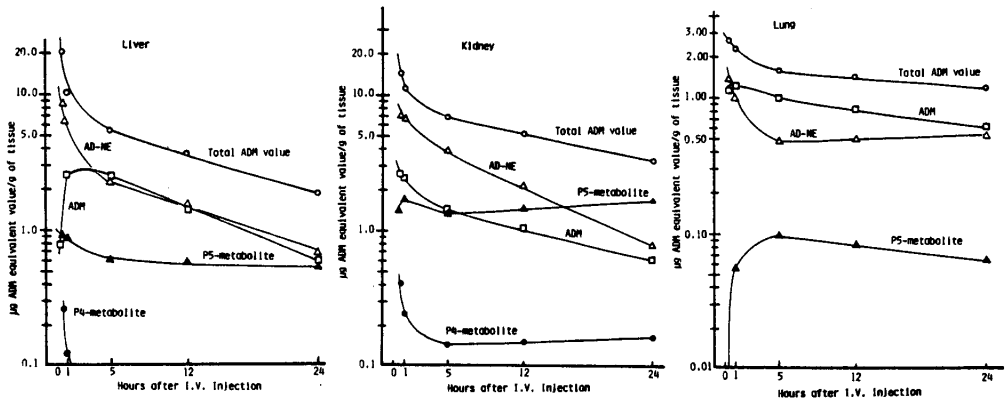

Fig. 2 Concentrations of ADM and its related fluorescent compounds in Ehrlich tumor bearing mouse tissues (Liver, Kidney and Lung) after a single intravenous injection (2.8 mg/kg), examined by HPLC. The values are expressed as the mean of 3 mice. Nomenclature of the figure is the same as Fig. 1.

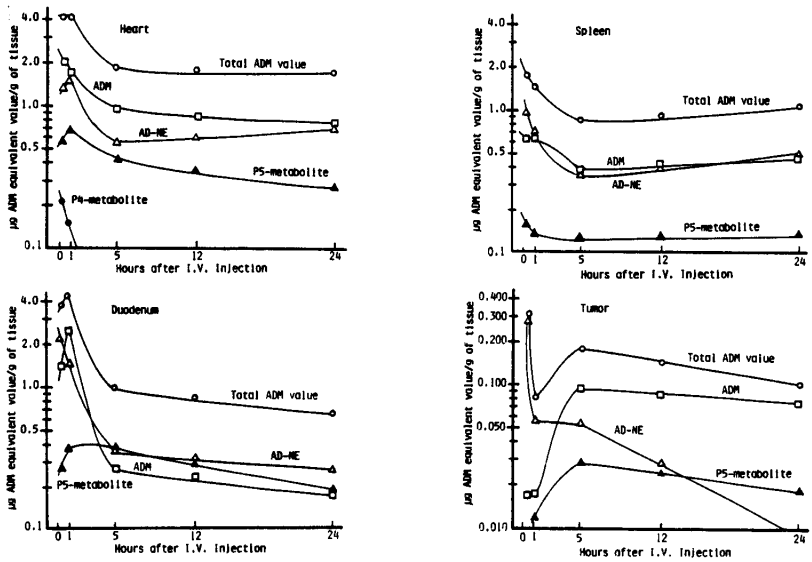

Fig. 3 Concentrations of ADM and its related fluorescent compounds in Ehrlich tumor bearing mouse tissues (Heart, Spleen, Duodenum and Tumor) after a single intravenous injection (2.8 mg/kg), examined by HPLC. The values are expressed as the mean of 3 mice. Nomenclature of the figure is the same as Fig. 1.

TABLE 1. THE PHARMACOKINETIC PARAMETERS OF ADM AND ITS RELATED FLUORESCENT COMPOUNDS IN EHRLICH TUMOR-BEARING MICE PLASMA AFTER THE ADMINISTRATION OF A SINGLE INTRAVENOUS INJECTION OF ADM (2.8 MG/KG)

	A (ng/ml)	α (h^{-1})	B (ng/ml)	β (h^{-1})	A+B (ng/ml)	V (L/kg)	K10 (h^{-1})	K12 (h^{-1})	K21 (h^{-1})	t·1/2 (h)	V · K10 clearance (L/kg/hr)
Total	560	3.5	130	0.026	690	4.1	0.13	2.7	0.67	27	0.5
AD-NE	360	3.5	80	0.020	440	6.4	0.11	2.7	0.64	34	0.7
ADM	300	4.6	57	0.062	357	7.8	0.36	3.5	0.79	11	2.9
AD-NOL	9	2.8	2	0.014	11	253.4	0.08	2.2	0.53	48	19.3

The parameters were obtained graphically (Fig 1) and calculated by the method of Riegelman et al. (1968). Total= total adriamycin equivalent values, AD-NE= total concentrations of P2 plus P3 metabolite, AD-NOL= adriamycinol

TABLE 2. THE RATIOS OF TISSUE PER PLASMA IN TOTAL ADM EQUIVALENT FLUORESCENT (T) AND ADM VALUES (ADM)

	Ratios of tissue per plasma													
Time after injection	Liver		Kidney		Heart		Spleen		Lung		Duodenum		Tumor	
	(T)	(ADM)	(T)	(ADM)	(T)	(ADM)	(T)	(ADM)	(T)	(ADM)	(T)	(ADM)	(T)	(ADM)
20 min	67.7	7.9	47.1	126.0	14.4	18.6	6.7	6.5	9.3	11.0	13.2	12.6	2.0	1.2
60 min	69.0	47.8	75.0	46.3	28.4	33.9	10.9	13.0	16.2	23.4	30.0	47.2	1.6	1.3
5 h	47.0	59.1	60.0	34.1	17.1	23.6	8.3	9.7	14.2	23.5	9.5	7.2	2.6	3.2
24 h	27.2	43.1	48.8	48.1	26.8	57.9	16.8	36.5	17.7	45.5	10.3	14.3	2.5	6.8

The values are expressed as mean values of 3 determinations

pound values detected in Ehrlich tumor-bearing mouse liver, kidney, heart, spleen, duodenum and tumor tissues. In the liver, the elimination curve of total ADM was similar to that of AD-NE. In the kidney, total ADM values registered at a high level being 3384 ng/g 24 h later, but the ADM value was 637 ng/g. The P5-metabolite value in kidney tissue gradually increased with time. In the lung and heart, total ADM and ADM values showed a slow rate of clearance, also AD-NE gradually increased with time. In the spleen, total ADM, AD-NE, ADM and P6-metabolite values gradually increased during the period between 5 and 24 h. In the duodenum, total ADM, AD-NE and ADM values showed the highest concentrations one h later, thereafter decreasing gradually during the next 24 h. In the tumor, ADM and its related fluorescent compounds all showed the lowest levels of all the examined tissues.

The ratios of tissue per plasma in total ADM (T) or ADM values (ADM) are shown in Table 2 with sacrificing times. In the heart, spleen, and lung, the ratios of ADM values are mostly much larger than those of total ADM values, therefore, ADM might have a stronger affinity with those than with its related fluorescent compounds. The ratios for total ADM and ADM values in the liver,

kidney and heart tissues showed a two-digit magnitude increase each time they were examined. At 24 h later, the ratios for the ADM value in the liver, kidney, heart, and lung tissues were high, being 43.1, 48.1, 57.9 and 45.5 times respectively. Furthermore, the ratios for total ADM values 20 min later in the spleen, lung and tumor tissues were comparatively small but 24 h later the ratios increased to 36.5, 45.5 and 6.8 times respectively.

DISCUSSION

Arena *et al.*, (5) first examined the tissue distribution of ADM, and reported that the concentration in most tissues was at least one or two orders of magnitude greater than that in blood. Similar results were reported thereafter; namely, high concentrations of ADM were shown in the liver, kidney, spleen and lung, and high tissue affinities in the heart and spleen tissues (5-7).

In the determination of the presence of ADM in the tissues, radioisotopic and fluorophotometric methods are used predominantly, but in doing so, related compounds are contaminated, therefore, there is usually some trouble determining the precise behavioral patterns of ADM. Few reports are found concerning tissue distribution of ADM differentiating the behavior of ADM from that of the metabolized form (7, 11).

For the identification of the metabolites of ADM, there are several opinions concerning the processes of contamination of aglycones and polar metabolite (containing adriamycinol) (4, 7, 9, 11). Benjamin *et al.*, (10) precisely examined the metabolites in human plasma, and reported that the pharmacokinetic pattern of ADM (in the unmetabolized form) differed from that of the total fluorescent compound value.

In our study, we examined the pharmacokinetics of ADM and its related fluorescent compounds in mouse plasma using a two-compartment open model, and found that the marked differences between ADM and total ADM or ADNE values lay in pharmacokinetic parameters. Furthermore, such differences were also shown in the tissue distribution. Therefore, these may reveal pharmacological variances on the site. As there is a wide difference between the value of K10 and β, it may be adequate to use the three-compartment open model in the pharmacokinetic analysis of ADM and its related fluorescent compounds by prolonging the administration time.

As for the interaction of ADM with biological components, it has been reported that ADM is intercalated with DNA and RNA proteins (14). It also influences the formation of a lipid-drug complex (15), enters into coenzyme Q related enzyme systems (16), and interacts with membrane lipid domains (17).

In the present experiment, high values for the ratios of tissue to plasma ADM were shown in the liver, heart, spleen and lung tissues even after 24 h, therefore it may be considered that, in these tissues, some unique interaction

with ADM or the transport system exists.

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