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DETERMINATION OF ADRIAMYCIN (DOXORUBICIN) AND RELATED FLUORESCENT COMPOUNDS IN RAT LYMPH AND GALL BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The concentrations of adriamycin (ADM) and related fluorescent compounds in lymph and gall were determined by high-performance liquid chromatography (HPLC) after a single intravenous injection into AH 109A tumour-bearing rats. HPLC was carried out by using Zorbax Sil as the stationary phase and chloroform–isopropanol–acetic acid–water–sodium acetate buffer (pH 4.5) (100:100:14:14:1) as the mobile phase, with a fluorescence spectrophotometric detector at an excitation wavelength of 470 nm and an emission wavelength of 585 nm. The detection limit for ADM was down to 1.0 ng/ml. In the thoracic duct lymph, the concentration of total ADM equivalent values (total ADM values) was maximal 30 min after injection and, after a subsequent decrease, increased gradually from 60 to 180 min. The ratio of total ADM in lymph to that in plasma at 180 min was 1.5 times that at 30 min. In gall, the total ADM showed a maximal level of 20.0 μg/ml at 30 min.

INTRODUCTION

Adriamycin (ADM) is an anthracycline antibiotic used extensively for the treatment of leukaemia and various malignant tumours[1]. Its initial half-life in plasma after intravenous injection is very short[2–5] and it has a strong affinity to tissue proteins[3,6–8] and deoxyribonucleic acid[9]. Its biliary excretion is the major excretory pathway in several animal species[10], but its passing into lymph has not yet been examined in detail.

In this study we devised a high-performance liquid chromatographic (HPLC) method for the determination of ADM and applied it to the transport of ADM into rat thoracic lymph and gall.

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EXPERIMENTAL

Reagents

Adriamycin hydrochloride, adriamycinone and adriamycinol were kindly donated by Farmitalia (Milan, Italy) and daunomycin was obtained from Meiji Seika (Tokyo, Japan). Chloroform and isopropanol were of HPLC grade. Acetic acid and sodium acetate were of analytical reagent grade.

Animal experiments

AH 109A ascites tumour (2×10^6 cells per animal) was inoculated on the back of Donryu male rats (body weight, *ca.* 160–170 g). The animals had free access to food and water. Seven days later, the rats were anaesthetized with diethyl ether, injected intravenously with 1.0 mg/kg of ADM or daunomycin (DUM) solution (1 mg/ml) with a syringe with a Harvard Apparatus infusion pump to the right inferior vena cava (0.53 ml/min), and the blood was collected with a heparinized syringe from the left inferior vena cava. The plasma was isolated. For studying the passing of ADM and DUM into lymph, the rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and the thoracic duct was cannulated according to the method of Bollman *et al.*[11]: a heparinized vinyl catheter (I.D. 0.5 mm; Imamura Rubber, Tokyo, Japan) was inserted into the duct and fixed with aid of a drop of Aron Alpha A tissue cement (Sankyo, Tokyo, Japan). The thoracic duct lymph was collected. For studying the passing of ADM into gall, the rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and the common bile duct was cannulated with a heparinized vinyl catheter. The gall was collected. Before or after these cannulations the rats were injected intravenously with 1.0 mg/kg of ADM or DUM solution (1 mg/ml) with a syringe with a Harvard Apparatus infusion pump to right inferior vena cava (0.53 ml/min).

Determination of ADM and related fluorescent compounds

ADM, related fluorescent compounds and DUM in the plasma, lymph and gall of AH 109A tumour-bearing rats were determined by modifying the previously reported HPLC method[12]. In brief, a Hitachi Model 635A high-performance liquid chromatograph was connected to a Hitachi Model 650-10S high-sensitivity fluorescence spectrophotometer, the results were recorded on a Hitachi Model 056 recorder and calculations were performed using a Hitachi Model 834-30 Chromato-Processor as an integrator, based on the ratio of the peak area to that of standard ADM or DUM (external standard method). The stationary phase was Zorbax Sil (5 μm) packed in a stainless-steel tube (150 × 4.6 mm I.D.). The mobile phase was chloroform–isopropanol–acetic acid–water–sodium acetate buffer (pH 4.5) (100:100:14:14:1) 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. ADM was extracted from the biological samples by the method described previously, using chloroform–methanol (4:1)[6]. All operations with ADM and related fluorescent compounds were carried out in near darkness.

RESULTS

The use of Zorbax Sil as the stationary phase and chloroform–isopropanol–acetic acid–water–sodium acetate buffer (pH 4.5) (100:100:14:14:1) as the mobile phase resulted in a good separation of ADM, adriamycinol, adriamycinone and DUM. A typical example of the HPLC analysis of rat gall is shown in Fig. 1. Peaks 1–7 (P1 metabolite–P7 metabolite) representing ADM-related compounds were detected. P2 was identified as adriamycinone and P6 as adriamycinol by HPLC and thin-layer chromatography[6]. The contaminating biological blank was detected at the same site as P5 but in trace amounts. The detection limit for ADM was down to 1.0 ng/ml. Tables I and II show the concentrations of ADM and related fluorescent compounds in AH 109A tumour-bearing rat plasma and thoracic duct lymph after a single intravenous injection (1 mg/kg), as determined by HPLC. AD–NE indicates the total concentration of P2 plus P3 metabolites. The values are expressed as means ± standard errors (ng adriamycin equivalent value/ml).

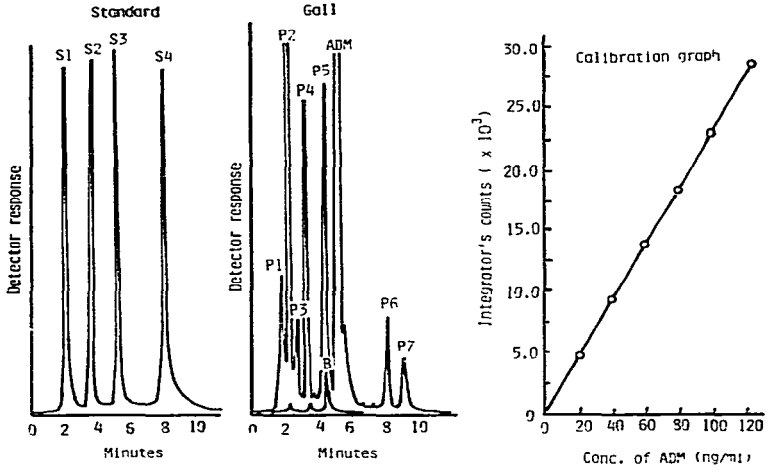

Fig. 1. High-performance liquid chromatogram of a standard sample mixture (standard), ADM-administered rat gall and calibration graph of adriamycin obtained with a Hitachi Model 635A high-performance liquid chromatograph with a Zorbax Sil column (150 × 4.6 mm I.D.) at room temperature. Mobile phase, chloroform–isopropanol–acetic acid–water–sodium acetate buffer (pH 4.5) (100:100:14:14:1); flow-rate, 1.0 ml/min; detector, Hitachi Model 650-10S fluorescence spectrophotometer (excitation, 470 nm; emission, 585 nm); S1 = adriamycinone; S2 = daunomycin; S3 = adriamycin; S4 = adriamycinol; P2 = adriamycinone; P6 = adriamycinol; ADM = adriamycin; B = gall blank.

ADM and related fluorescent compounds were eliminated rapidly from plasma, but in the thoracic duct lymph the concentration of AD–NE and the total ADM equivalent fluorescent values (total ADM values) showed maxima 30 min after injection and, after a subsequent decrease, increased gradually from 60 to 180 min. The total ADM values in plasma and thoracic duct lymph are shown in Fig. 2 in comparison with the values of total DUM equivalent fluorescent values (total DUM values). Total DUM values in the plasma showed were maximal at 5 min and decrease rapidly from 5 to 30 min, then they are almost constant up to 360 min. Total

TABLE I

CONCENTRATIONS OF ADRIAMYCIN AND RELATED FLUORESCENT COMPOUNDS IN AH 109A TUMOUR-BEARING RAT PLASMA AFTER A SINGLE INTRAVENOUS INJECTION

The values are means ± S.E. for at least eight animals.

Time after injection (min)	*Substance (ng adriamycin equivalent value/ml)*					
	*AD-NE**	*P4 metabolite*	*P5 metabolite*	*Adriamycin*	*Adriamycinol*	*Total*
5	211.29 ± 39.42	9.29 ± 2.73	35.31 ± 12.93	159.82 ± 80.32	5.07 ± 1.59	418.11 ± 113.69
30	100.12 ± 14.72	13.49 ± 1.31	21.56 ± 9.70	24.40 ± 9.31	2.13 ± 0.23	168.19 ± 25.20
180	65.39 ± 9.84	6.80 ± 1.38	25.47 ± 10.97	16.37 ± 3.36	1.53 ± 0.44	113.00 ± 16.72
360	48.01 ± 13.26	6.99 ± 2.95	23.45 ± 8.07	8.36 ± 2.97	1.41 ± 0.62	84.56 ± 14.76

* AD-NE = P2 metabolite plus P3 metabolite.

TABLE II

CONCENTRATIONS OF ADRIAMYCIN AND RELATED FLUORESCENT COMPOUNDS IN AH 109A TUMOUR-BEARING RAT THORACIC LYMPH AFTER A SINGLE INTRAVENOUS INJECTION

The values are means ± S.E. for at least five animals.

Time after injection (min)	*Substance (ng adriamycin equivalent value/ml)*					
	*AD-NE**	*P4 metabolite*	*P5 metabolite*	*Adriamycin*	*Adriamycinol*	*Total*
30	532.95 ± 247.71	5.55 ± 4.22	58.24 ± 53.74	104.06 ± 35.59	3.51 ± 1.78	704.29 ± 329.19
60	271.49 ± 84.98	4.88 ± 2.03	31.88 ± 25.16	92.25 ± 33.65	3.77 ± 1.35	404.37 ± 123.53
90	394.77 ± 179.44	5.08 ± 2.54	9.05 ± 4.17	57.63 ± 24.62	3.76 ± 1.18	470.28 ± 183.96
120	390.46 ± 152.46	2.84 ± 2.37	10.57 ± 5.20	112.18 ± 80.03	3.31 ± 1.13	519.36 ± 216.64
150	447.30 ± 102.79	1.55 ± 1.34	3.66 ± 1.91	67.51 ± 27.52	3.86 ± 1.95	539.88 ± 106.97
180	647.84 ± 300.64	2.54 ± 1.71	11.05 ± 5.32	114.14 ± 65.62	2.53 ± 1.42	779.71 ± 322.15
0–180	468.33 ± 105.78	4.35 ± 1.54	19.24 ± 13.60	94.54 ± 38.21	3.28 ± 0.49	589.74 ± 130.10

* AD-NE = P2 metabolite plus P3 metabolite.

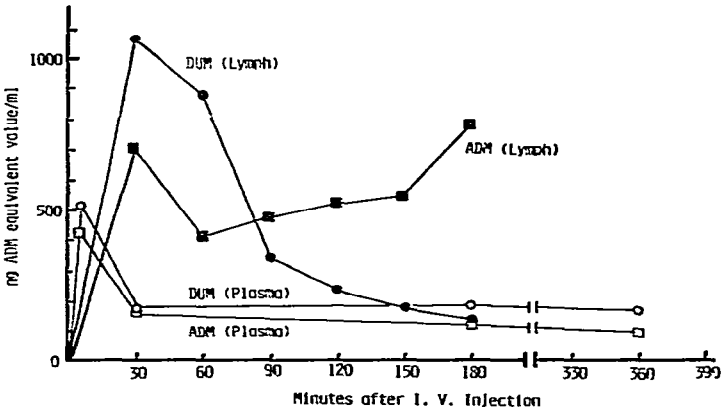

Fig. 2. Concentrations of total adriamycin (ADM) and daunomycin (DUM) (equivalent values) in AH 109A tumour-bearing rat plasma [ADM (plasma) and DUM (plasma)] and thoracic duct lymph [ADM (lymph) and DUM (lymph)] after a single intravenous injection, examined by HPLC. The values are means for at least five animals.

DUM values in the rat thoracic duct lymph differed from the ADM value, showing a maximum at 30 min, then decreasing gradually to near to the its plasma value at 180 min.

Table III shows the ratios of ADM and related compounds in lymph to those in plasma 30 and 180 min after injection: the AD–NE, ADM and total ADM values at 180 min were up to 1.5 times the corresponding values at 30 min.

The passing of ADM into rat gall is illustrated in Fig. 3. The total ADM level was maximal at 30 min (20.0 μg/ml), being rich in ADM (unchanged form), and thereafter decreased gradually up to 120 min. At 450 min, the level was still (5.0 μg/ml).

DISCUSSION

We have previously reported a method for the determination of ADM in serum and tissues with Zorbax Sil as the stationary phase and 3.8% sodium acetate in isopropanol as the mobile phase[6,12]. However, there were problems with broad chromatographic peaks and the long analytical time (22 min). In this study, we devised a method based on these points that gave sharp chromatographic peaks, with a rapid (10 min) and highly sensitive determination of ADM.

For the identification of metabolites of ADM, opinions vary about contamination of aglycones and polar metabolites (containing adriamycinol)[3,5,6,13–15], but in some work no metabolites were detected[2]. In our experiments, the main metabolites in rat plasma, lymph and gall were adriamycinone, P4 metabolite. P5 metabolite and adriamycinol, and other minor metabolites were also detected.

ADM was shown to be passed into lymph or gall in high concentrations. Because lecithin and bile acid, the main constituents of gall, have been related to the passing of drugs into the lymph[16], a high concentration of ADM in lymph was considered to be correlated with its high excretion into gall.

Arena *et al.*[7] first reported on the pharmacokinetics of ADM in mice, stating that the concentrations in most tissues were at least one or two orders of magnitude

TABLE III

RATIOS OF ADRIAMYCIN AND RELATED FLUORESCENT COMPOUNDS IN LYMPH TO THOSE IN PLASMA FROM AH 109A TUMOUR-BEARING RATS AFTER A SINGLE INTRAVENOUS INJECTION

The values are means of at least five determinations.

Time after injection (min)	*Ratio of lymph to plasma levels*					
	*AD-NE**	*P4 metabolite*	*P5 metabolite*	*Adriamycin*	*Adriamycinol*	*Total*
30	5.32	0.41	2.70	4.26	1.65	4.19
180	9.91	0.37	0.43	6.97	1.65	6.90

* AD-NE = P2 metabolite plus P3 metabolite.

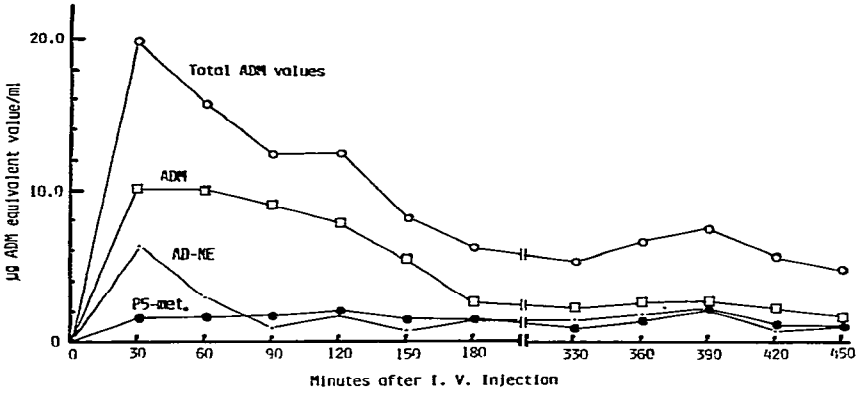

Fig. 3. Concentrations of adriamycin and related fluorescent compounds in AH 109A tumour-bearing rat gall after a single intravenous injection. Total ADM values = total adriamycin equivalent values; ADM = adriamycin; AD–NE = P2 metabolite plus P3-metabolite; P5-met. = P5 metabolite. The values are means for at least three animals.

greater than blood levels, and demonstrated that biliary excretion was the primary route of elimination. The high retention of ADM in tissues in various animals has been reported[3,6–8]. For this reason, it is considered that ADM is structurally similar to quinone compounds present in animal tissues, and can enter coenzyme Q and related enzyme systems[17] or has an especially strong affinity to deoxyribonucleic acid[9] and some negatively charged phospholipids[18].

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