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Marked Inter-Patient Variation in Adriamycin Biotransformation to 7-Deoxyaglycones: Evidence from Metabolites Identified in Serum

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Abstract—*Several factors are known to modulate the clinical pharmacokinetics of adriamycin (ADR). Biotransformation has not been studied in this context because of problems identifying serum metabolites. We have studied patterns of ADR biotransformation in 25 patients with normal liver and kidney function and in most cases receiving ADR for the first time. Three major serum metabolites were identified by HPLC, TLC and mass spectrometry and their pharmacokinetics were followed over a 24-hr period. The relative amount of each metabolite present in a patient was quantitated by calculating its AUC. Adriamycinol was the major metabolite detected in the majority of patients. Adriamycin 7-deoxyaglycone was detected in the serum of 15 patients where it accounted for a small percentage of the total ADR concentration (1–5%). Its apparent half-life was normally less than 30 min. Adriamycinol 7-deoxyaglycone was detected in the serum of only 13 patients where it accounted for a greater percentage of the total ADR concentration (10–20%). Its pharmacokinetics exhibited marked inter-patient variations, with apparent half-lives ranging from 0.1 to 24 hr. There was a correlation between the AUC of ADR and the relative amount of metabolites present in each patient ($r = 0.73$). Thus, biotransformation may explain, partly, inter-patient variations in ADR pharmacokinetics. In turn, variations in biotransformation are dictated by whether or not ADR is converted to 7-deoxyaglycones.*

INTRODUCTION

ADRIAMYCIN (ADR, **1** Fig. 1) is a naturally occurring anti-biotic anticancer drug possessing a broad spectrum of activity in a variety of human solid malignancies and leukemias [1]. Specific cardiotoxicity limits its administration in the clinic to a recommended safe cumulative dose of 550 mg/m^2 [2]. However, significant differences are observed in the level of drug exposure at which objective evidence of heart failure is detected [3]. Continuous infusion of ADR can ameliorate cardiotoxicity, indicating that the early high peak levels achieved after an i.v. bolus injection are the most detrimental to the heart [4]. However, attempts to correlate drug levels with toxicity and also therapeutic response have been mostly unsuccessful because of inherently large inter-patient variations in the pharmacokinetics of ADR [5–8]. Several factors have been identified which could contribute to inter-patient variations in handling of ADR. These include age, dose, concomitant treatment, prior exposure to the drug and liver function status [5, 9–12]. Metabolism of the drug has not been studied in this context. In this report we present findings of a marked inter-patient variation in the biotransformation of ADR to 7-deoxyaglycone metabolites.

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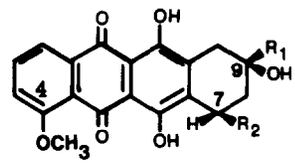
MATERIALS AND METHODS

Patients

A total of 25 subjects receiving ADR as part of combination chemotherapy for advanced malignant disease were studied. Samples were taken from 18 after the first course of ADR treatment (concomitant chemotherapy was administered a day later); from six after the second course of ADR treatment (administered together with 500 mg/m^2 cyclophosphamide); and from one after six previous courses of ADR treatment (in combination with 5-fluorouracil and mitomycin-C). The dose of ADR was either 30 or 40 mg/m^2. No patient had



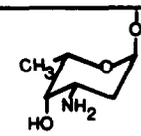
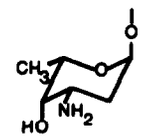
COMPOUND	R_1	R_2
1 ADR	$-\overset{\overset{O}{\|}}{\underset{13}{C}}-CH_2OH$	
2 AOL	$-\underset{H}{\overset{OH}{\underset{\|}{\overset{\|}{C}}}}-CH_2OH$	
3 ADR-ONE	$-\overset{\overset{O}{\|}}{C}-CH_2OH$	OH
4 AOL-ONE	$-\underset{H}{\overset{OH}{\underset{\|}{\overset{\|}{C}}}}-CH_2OH$	OH
5 ADR-DONE	$-\overset{\overset{O}{\|}}{C}-CH_2OH$	H
6 AOL-DONE	$-\underset{H}{\overset{OH}{\underset{\|}{\overset{\|}{C}}}}-CH_2OH$	H

Fig. 1. The structure of adriamycin and the reference compounds used as metabolite standards for both HPLC and TLC assays.

severe liver or kidney dysfunction and none were heavy drinkers of alcohol. More complete information on each patient is to be found in Table 1. Blood samples (10 ml aliquots) were withdrawn through indwelling catheters (positioned in the arm opposite to that used for drug injection) before treatment and at 5, 10, 15, 20, 30, 60 min, 2, 4, 6, 8, 10, 12 and 24 hr after an i.v. bolus push of ADR lasting for 3 min. Exact times of blood sampling were recorded. After blood was collected it was allowed to clot in plain glass tubes for 1 hr at 4°C. Sera were then separated and stored at −20°C. Prior to extraction sera were thawed at room temperature.

Chemicals and metabolite reference standards

All methanol, chloroform and propan-2-ol and all HPLC solvents were of HPLC reagent grade (Fisons Scientific Apparatus, Loughborough, U.K.) and all TLC solvents were of analytical reagent grade (AnalaR, BDH Chemicals Ltd., Poole, U.K.). Water was de-ionised and double distilled in a quartz glass still. Pure ADR-HCl, adriamycinol (AOL, **2** Fig. 1), ADR 7-hydroxyaglycone (ADR-ONE, **3** Fig. 1) and ADR 7-deoxyaglycone (ADR–DONE, **5** Fig. 1) were a gift from Dr. S Penco (Farmitalia, Milan, Italy). ADR–ONE and AOL 7-hydroxyaglycone (AOL–ONE, **4** Fig. 1) were synthesised by mild acid hydrolysis with 0.1M HCl at 55°C. ADR–DONE and AOL 7-deoxyaglycone (AOL–DONE, **6** Fig. 1) were synthesised by catalytic hydrogenation using a poisoned palladium catalyst [13,14]. Purity of the four synthesised aglycones has already been reported elsewhere [15]. Daunorubicin–HCl (DNR), the internal standard, was from May & Baker Ltd. (Dagenham, U.K.)

High performance liquid chromatography

HPLC was performed throughout using an Altex model 110A pump and an Altex model 210 injection port with a 20 μl injection loop (Beckman–RIIC, High Wycombe, U.K.); a Gilson spectro-glo filter fluorimeter with narrow band interference filters at 480 nm (excitation) and 560 nm (emission) and a 10 μl quartz micro-flow cell (Gilson, Villiers-le-Bel, France); and a Shimadzu CR-1B computing integrator (supplied by Scotlab Instrument Sales, Bellshill, U.K.). The stationary phase was μ-Bondapak C18 (Waters, Northwich, U.K.). It was packed into 250 mm by 4.6 mm i.d. stainless steel columns using a Shandon HPLC column packer (Shandon, Runcorn, U.K.) as previously described [16]. Assay 1 used a mobile phase that consisted of 5mM phosphoric acid (62.5%), methanol (15%), acetonitrile (15%) and propan-2-ol (7.5%) pH 3.2. Elution was isocratic at a flow rate of 2 ml/min. Assay 2 used a mobile phase that consisted of 5mM-phosphoric acid (74%) and propan-2-ol (24%), pH 3.2. Elution was isocratic at a flow rate of 1.2 ml/min. A Gilson Microcol TDC 80 fraction collector was used for preparative HPLC.

Quantitation

ADR and metabolite concentrations in sera samples were quantitated using a computing integrator. Calibration of the integrator was by a two point external standard method with 10 replicate methanolic solutions containing at a concentration of either 1 μg/ml or 10 μg/ml ADR, reference metabolites and DNR. Calibration was repeated regularly. The limit of detection of both assays in serum was 3 ng/ml for ADR and 1 ng/ml for reference metabolites. Standard calibration curves of ADR, reference metabolites and DNR were linear over the concentration range 25 ng/ml–10 μg/ml (0.5–200 ng injected onto the HPLC column). None of the extracted patient samples fell out with this range of concentrations.

Thin layer chromatography

TLC separations were performed with 20 × 20 cm or 20 × 10 cm glass plates coated with

Table 1. Inter-patient variations in the metabolism of adriamycin: the patient group studied

Patient	Sex	Age	Weight (kg)	Primary tumour	Liver metastases	Prior ADR exposure (mg/m^2)	Dose (mg/m^2)	Liver function Alb+ *36–50g/L	Liver function Bil‡ 3–18 μmol/L	Liver function AST** 10–35μ/L	Kidney function CT++ 60–110 μmol/L	Kidney function Urea 2.5–7 mmol/L
JJ	F	50	76	Breast ca.	No	nil	40	41	5	36	67	3.2
MB	"	56	62	Breast ca.	"	"	"	41	5	14	83	4.5
JM	"	47	81	Breast ca.	"	"	"	38	7	27	91	4.5
MMcC	"	54	70	Breast ca.	"	"	"	30	9	34	67	3.3
AG	"	57	44	Gastric ca.	"	"	30	35	4	20	63	3.8
MF	"	42	62	Ovarian ca.	"	"	40	29	3	14	58	3.8
MMcL	"	68	42	Unknown adenocarcinoma	Yes	"	30	32	20	18	82	5.0
WMcG	M	64	52	Gastric ca.	No	"	"	35	5	30	90	9.1
EB	"	50	56	Gastric ca.	"	"	"	37	9	16	83	4.3
AMcD	"	51	72	Gastric ca.	"	"	"	43	7	17	95	5.9
JL	"	65	55	Gastric ca.	Yes	"	"	27	5	15	94	6.8
JH	"	75	52	Gastric ca.	"	"	"	45	17	46	98	5.8
CL	"	62	54	Gastric ca.	"	"	"	35	6	36	56	4.1
RR	"	53	68	Unknown adenocarcinoma	No	"	"	36	5	15	109	6.1
JU	"	59	62	Unknown adenocarcinoma	Yes	"	"	37	8	99	75	4.6
WM	"	58	85	Hepatocellular ca.	No	"	40	42	13	38	70	5.2
IC	"	58	93	Adrenal ca.	"	"	30	27	12	15	80	8.0
JH	"	35	80	S.C. lung ca.	"	"	40	40	12	21	95	4.9
LMcG	F	29	46	S.C. lung ca.	No	40	40	41	7	12	55	4.7
AA	"	34	53	S.C. lung ca.	"	"	"	38	8	17	75	5.7
WG	"	69	74	Lymphoma	"	30	30	29	16	28	150	—
MK	"	59	75	Lymphoma	"	"	"	35	7	17	80	3.9
AB	M	79	67	Lymphoma	"	"	"	30	15	17	140	4.2
MG	"	80	65	Lymphom	"	"	"	38	14	30	115	5.2
TA	"	45	64	Gastric ca.	"	180	"	42	14	23	82	5.4

*Normal range of value. +Albumin; ‡Bilirubin;

**Aspartate transaminase; ++Creatinine

Data are presented principally on liver function status, dose and prior exposure of drug — all these being factors which are known to alter the disposition of adriamycin

a 250 μm layer of silica gel G (Analtech Inc., Newark, U.S.A.). Three different ascending solvent systems were used. S1 was chloroform : methanol : acetic acid : water (80 : 20 : 14 : 6), S2 was chloroform : methanol : water (80 : 20 : 3) and S3 was ethyl acetate : ethanol : acetic acid : water (80 : 10 : 5 : 5) [14]. ADR, its metabolites and DNR were visualised as orange fluorescent spots under u.v. light at 254 nm. Metabolites isolated from patient serum by preparative HPLC were reconstituted in 10 μl of methanol and applied directly onto the plate.

Mass spectrometry

A mass spectrum of methanolic solutions of AOL–DONE isolated from patients MB and JJ was obtained by direct probe injection mass spectrometry. The mass spectrometer was a Kratos model MS 9025 and was controlled by a DS 55C data system (Kratos Analytical Instruments, Manchester, U.K.). It was set at 500 μA and 70 eV and source temperature was 220°C.

Rapid extraction of ADR and metabolites from serum (and urine)

Rapid extraction was achieved by mixing 1 or 2 ml serum or urine with 5 vol. of chloroform : propan-2-ol (2 : 1) for a short period of time (2 × 2 min vortexing or 30 min shaking). Care was taken when mixing to avoid formation of a gel which greatly reduced extraction efficiency. DNR (50 or 100 ng) was added to samples as an internal standard before extraction. After extraction, samples were centrifuged at 2000 ***g*** for 15 min and two phases were separated. The upper aqueous phase was discarded by aspiration, the lower organic phase was evaporated to dryness [15]. The dry extracts were reconstituted in either 40, 50 or 100 μl of methanol immediately prior to HPLC. Rapid extraction recovers ADR and metabolites with efficiency of greater than 77% [15].

Mathematical analysis

Area under serum ADR and metabolite concentration/time curves (AUC), from zero to 24 hr, was calculated by the trapezoidal rule. Metabolite half lives were calculated by non-linear regression data analysis.

Identification of metabolites in patient serum

Serum metabolites were identified by comparing their chromatographic mobility against that of chemically pure reference metabolites. For identification by HPLC two mobile phases were employed, each of which yields a different elution profile of ADR and metabolites [15,16]. For identification by TLC three solvent systems were employed, each of which yields different R_f values for ADR and metabolites. AOL–DONE isolated from patients JJ and MB by preparative HPLC was analysed by mass spectrometry.

RESULTS

In a series of preliminary experiments blood was incubated with ADR to determine whether or not metabolism or chemical degradation could occur *in situ* during sample preparation. It has been claimed that whole blood can metabolise ADR to AOL [17]. Fresh blood was obtained from healthy volunteers. Whole blood, serum or plasma was incubated with 1 μg/ml ADR at 25°C for 1 hr, extracted and then analysed by HPLC. Results obtained from several replicate experiments showed no evidence of presence of fluorescent species other than ADR itself (Fig. 2a). All the metabolite standards used in this study do not degrade into fluorescent products which could be mistaken for additional metabolites. No endogenous peaks were extracted from pre-treatment sera which interfered with the identification of ADR and reference metabolites (Fig. 2b). Cyclophosphamide, which does not fluoresce, also did not interfere with the HPLC assays.

The largest number of metabolites separated from serum by HPLC was seven (Fig. 2c). Three were identified as: the alcoholic glycoside, AOL; the 7-deoxyaglycone of ADR, ADR–DONE; and the 7-deoxyaglycone of AOL, AOL–DONE. Two of the unidentified metabolites eluted earlier than AOL (peaks b and c, Fig. 2c); indicating that they were more water soluble than AOL and, therefore, most probably conjugates. The other two unidentified metabolites eluted later than ADR–DONE (peaks i and j, Fig. 2c); indicating that they were less water soluble than ADR–DONE and, therefore, most probably aglycones. The HPLC peak corresponding to AOL–DONE was isolated from patients MB and JJ and analysed by mass spectrometry. AOL–DONE has a mol. wt. of 400 and the mass spectrum of the isolated metabolite (Fig. 3) yielded a molecular ion m/z 400 and a recognisable fragmentation pattern (m/z 364, M $-2H_2O$; m/z 339, M $-CHOH$, $-CH_2OH$). Of all the seven metabolites detected, normally only AOL and AOL–DONE persisted in serum several hours after drug administration (Fig. 2d).

The pattern of adriamycin biotransformation within the whole group of patients studied

Unidentified conjugates were detected in the serum of 11 subjects and unidentified aglycones in the serum of only two. All four metabolites were at their peak concentration at the earliest time of blood sampling (5 min) and were no longer detectable by 1 hr. Accurate quantitation of unidentified metabolites is impossible because ADR and its

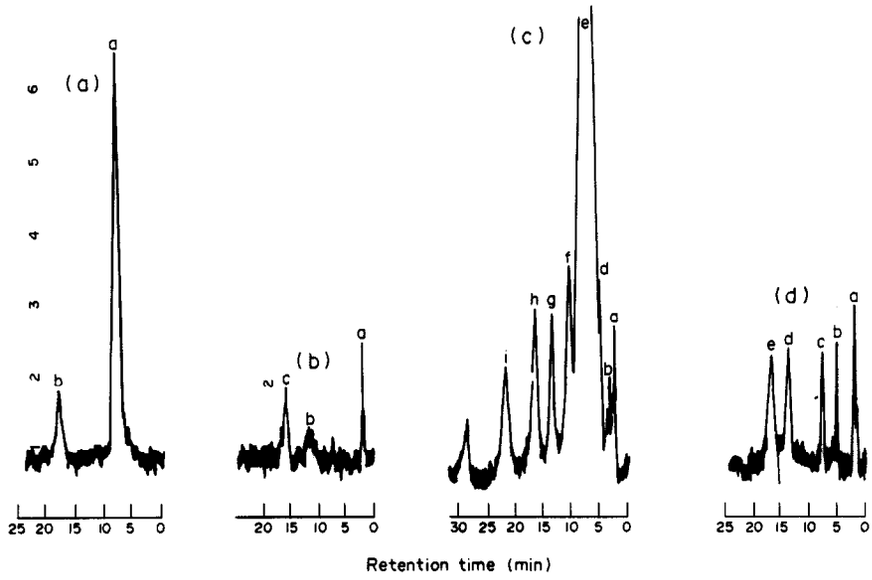


Fig. 2. Identification of adriamycin and its metabolites in patient serum by HPLC. Fluorescence detector attenuation was identical in all four chromatograms. (a) *Whole blood incubated with* 1 μg/ml *ADR for* 1 hr *at* 25°C. *Peaks are: a, ADR and b, DNR (internal standard).* (b) *Pre-treatment serum (MB). Peaks are: a and b, endogenous components; and c, DNR.* (c) *Serum* 5 min *after* 40 mg/m^2 *ADR i.v. (MB). Peaks are: a, endogenous components; b and c unidentified polar metabolites; d, AOL* (27 ng/ml); *e, ADR* (1.8 μg/ml); *f, AOL-DONE* (33 ng/ml); *g, DNR; h, ADR-DONE* (33 ng/ml); *and i and j, unidentified non-polar metabolites.* (d) *Serum* 4 hr *after drug administration (MB). Peaks are: a, endogenous components; b, AOL* (7 ng/ml); *c, ADR* (12 ng/ml); *d, AOL-DONE* (9 ng/ml) *and e, DNR.*

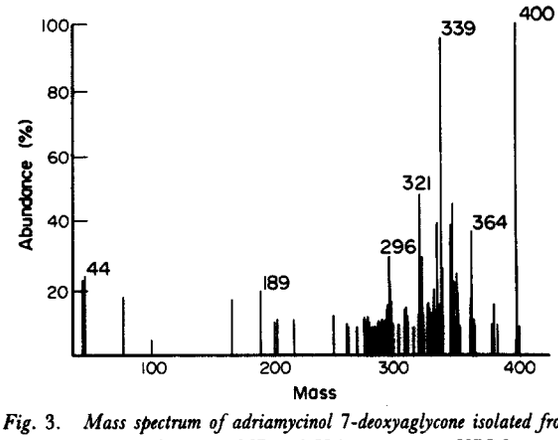


Fig. 3. Mass spectrum of adriamycinol 7-deoxyaglycone isolated from the serum of patients MB and JJ by preparative HPLC.

different metabolites do not fluoresce with the same efficiency [15]. However, if one assumes that the two conjugates fluoresce with the same efficiency as AOL and the two aglycones with the same efficiency as ADR–DONE, then their combined AUCs never amounted to more than 3% of the total ADR concentration present in serum.

The pharmacokinetics of the metabolites identified in the 25 patients studied are summarised in Table 2. The relative amount of each metabolite present in the serum of a patient was quantitated by calculating its AUC. Individual AUCs were expressed as a percentage of the total AUC of ADR and all metabolites detected in a patient's serum.

The distribution of ADR and identified metabolite AUC values within the whole group of patients studied are shown in Figs. 4–7. In Figs. 4–7 are also the distributions of AUC expressed as a percentage of total AUC. Figure 4 illustrates that even although ADR was administered at a narrow dose range (30 or 40 mg/m^2) its AUC varied from patient to patient by a factor of at least 5-fold. The distribution resembled that of a normal distribution. There were no significant differences in AUC after administration of the drug at the two different doses or in combination with cyclophosphamide(Student's *t*-test). Other factors must have been responsible for the inter-patient variation. Unchanged ADR accounted for, in excess of, 60% of the total serum concentration. AOL was detected in the serum of all the patients studied and in 21 patients was the major metabolite. The distribution of its AUC resembled that of a log normal distribution with a slight skew towards higher values (Fig. 5). AOL accounted for 15–20% of the total serum concentration. ADR–DONE was not detected in the serum of 10 patients (Table 2). In the other 15 patients the distribution of its AUC showed a narrow range with decreasing likelihood towards higher values (Fig. 6). When present, it accounted for less than 5% of the total serum concentration. In contrast, AOL–DONE which was not detected in the serum of 12 patients, accounted for up to 20% of the total serum concentration. In four patients it was the major metabolite. The distribution of its AUC showed a large spread in values (Fig. 7).

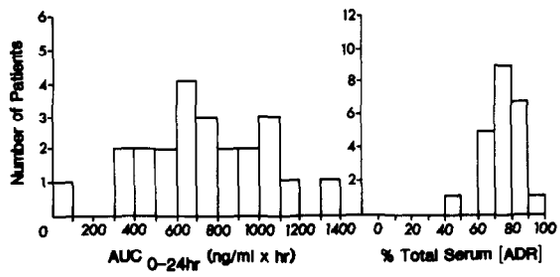


Fig. 4. Distribution of serum adriamycin within the whole group of patients studied. Results are expressed as the AUC in the left hand figure and as a percentage of the total serum concentration in the right hand figure.

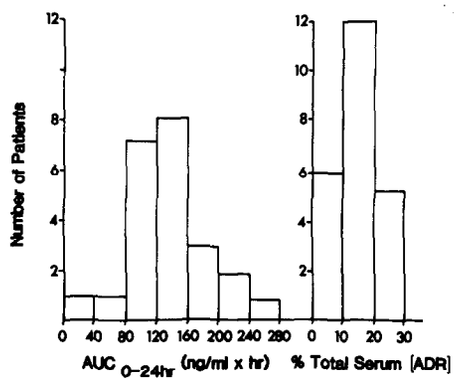


Fig. 5. Distribution of serum adriamycinol within the whole group of patients studied (AUC, left figure and percentage total, right figure).

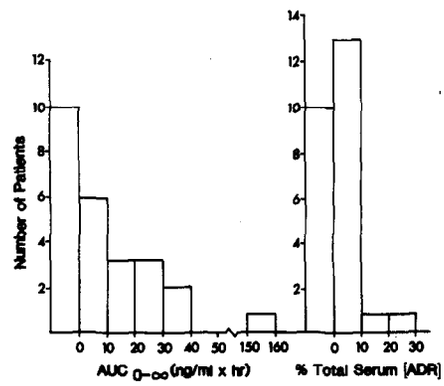


Fig. 6. Distribution of serum adriamycin 7-deoxyaglycone within the whole group of patients studied (AUC, left figure and percentage total, right figure). When the metabolite was not detected a value of zero was ascribed to AUC and percentage total.

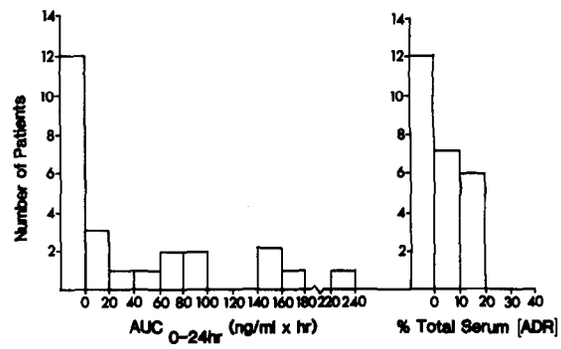


Fig. 7. Distribution of adriamycinol 7-deoxyaglycone within the whole group of patients studied (AUC, left figure and percentage total, right figure). When the metabolite was not detected a value of zero was ascribed to AUC and percentage total.

Pharmacokinetics of adriamycin metabolites

The pharmacokinetics of AOL were qualitatively similar in all patients studied. Peak serum concentrations always occurred during the first 20 min after drug administration and ranged from 16 to 807 ng/ml. However, they rarely exceeded 100 ng/ml (Table 2). Apparent half-lives ranged from 15 to 56 hr (Table 2). The pharmacokinetics of ADR–DONE were also qualitatively similar in the patients in which it was identified. Peak serum concentrations also occurred during the first 20 min after drug administration and ranged from 10 to 88 ng/ml (Table 2). In five patients its peak serum concentration was greater than that of AOL. Apparent half-lives were usually less than 30 min (Table 2). The main difference between AOL and ADR–DONE pharmacokinetics was apparent half-life. Examples of ADR–DONE serum profiles from three patients are in Fig. 8. The pharmacokinetics of AOL–DONE were not qualitatively similar in the patients in which it was identified. In four patients peak serum concentrations (5–23 ng/ml, Table 2) occurred 4–8 hr after drug administration and in nine patients peak serum concentrations (5–110 ng/ml, Table 2) occurred during the first hour after drug administration. Apparent half-lives were intermediate in value between those of AOL and ADR–DONE and ranged from 0.1 to 24 hr (Table 2). Serum profiles highlighting the large spread in peak levels of AOL and AOL–DONE are in Figs. 9 and 10.

Correlation between adriamycin pharmacokinetics and degree of biotransformation

Degree of biotransformation was defined as a percentage ratio as follows:

$$\frac{\Sigma\,\mathrm{AUC_{metabolites}}}{\mathrm{AUC_{Adriamycin}} + \Sigma\,\mathrm{AUC_{metabolites}}} \times 100.$$

In order to obtain a correlation plot with a positive

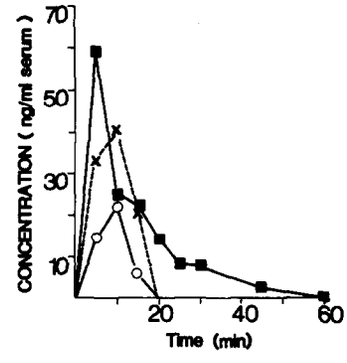


Fig. 8. Serum profiles of adriamycin 7-deoxyaglycone in patients TA, ■——■; MB, ×----× and AMcD, ○——○.

Table 2. Inter-patient variations in the metabolism of adriamycin: metabolite pharmacokinetic data from individual patients

Patient	ADR‡ AUC* ng/ml × hr	AOL‡ AUC* ng/ml × hr	AOL‡ PSC† ng/ml	AOL‡ Time to PSC (min)	AOL‡ Half-life (hr)	AOL–DONE‡ AUC* ng/ml × hr	AOL–DONE‡ PSC† ng/ml	AOL–DONE‡ Time to PSC (min)	AOL–DONE‡ Half-life (hr)	ADR–DONE‡ AUC§ ng/ml × hr	ADR–DONE‡ PSC† ng/ml	ADR–DONE‡ Time to PSC (min)	ADR–DONE‡ Half-life (hr)
JJ	454	144	807	5	16	76	93	15	21	3	22	15	0.3
MB	682	160	26	15	19	59	110	15	2	12	40	10	0.2
JM	782	187	21	15	28	88	19	5	15		ND		
MMcG	1108	129	73	5	22	39	5	480	10	35	34	15	0.4
AG	1328	140	18	15	36		ND‖				ND		
MF	1075	103	37	5	38	240	23	360	7	39	62	5	24
MMcL	674	143	16	5	17	148	7	40	13	20	37	10	0.3
WMcG	444	160	40	5	56		ND				ND		
EB¶	417	45	16	5			ND				ND		
AMcD	719	101	38	10	43		ND			9	21	10	0.2
JL	978	94	59	5	16		ND				ND		
JH	397	159	106	5	38	180	26	60	24	152	88	20	42
CL	1059	229	42	5	31	9	9	30	1	23	38	15	1
RR	774	65	23	5	26	70	16	10	12	27	33	10	0.6
JU	573	168	40	5	41	89	8	240	6	5	10	5	0.1
WM	869	260	92	5	52	145	21	240	5	6	10	15	0.4
IC	626	188	32	10	40		ND				ND		
JH	326	110	39	5	16		ND				ND		
LMcG	841	99	31	5	18		ND				ND		
AA	842	185	63	5	19		ND			5	15	15	0.2
WG	429	121	40	5	22	6	30	5	0.1	6	30	5	0.1
MK	906	229	56	5	31		ND				ND		
AB	542	115	82	5	16		ND				ND		
MG	68	11	26	5	2	23	51	5	1	23	51	5	0.7
TA¶	808	41	84	5			ND			12	59	5	0.2

*AUC_{0-24} hr;

†peak serum concentration;

‡chemical structures to be found in Figure 1

§$AUC_{0-\infty}$;

‖not detectable

¶incomplete blood sampling to determine half lives of AOL and AUC_{0-24} of ADR. This data is not included in distributions (Figs. 4 and 5).

slope, the AUC of ADR was plotted against:

$$\frac{AUC_{Adriamycin}}{AUC_{Adriamycin} + \Sigma AUC_{metabolites}} \times 100.$$

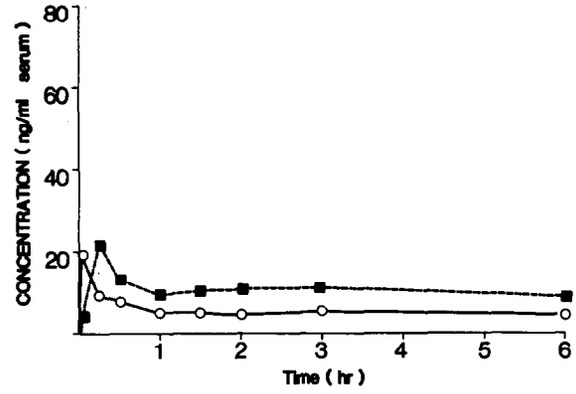


Fig. 9. Serum profiles of adriamycinol, ■----■ and adriamycinol 7-deoxyaglycone, ○——○ in patient JM.

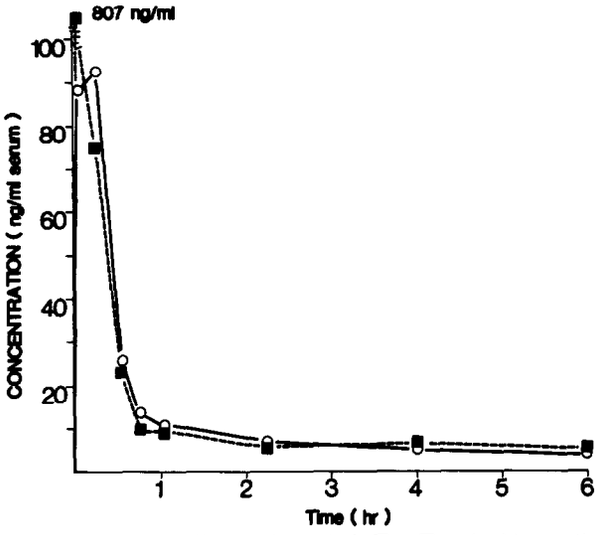


Fig. 10. Serum profiles of adriamycinol, ■----■ and adriamycinol 7-deoxyaglycone, ○——○ in patient JJ. Patient JJ was one of only two patients in the whole group to develop signs of ADR-induced cardiotoxicity.

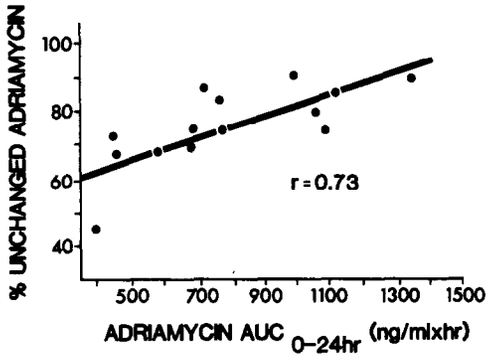


Fig. 11. Correlation between the AUC of adriamycin and the relative amount of metabolites present in serum. For a definition of degree of metabolism refer to results section.

The plot had a regression correlation coefficient of 0.73 and shows the relationship between ADR pharmacokinetics and biotransformation (Fig. 11). When the AUC of ADR was lower a relatively greater amount of metabolites were present in serum. When the AUC of ADR was higher a relatively smaller amount of metabolites were present in serum.

The pattern of urinary excretion of adriamycin and its metabolites

In urine only AOL, AOL–DONE and ADR–DONE were detected (Table 3). However, the HPLC assays were unable to distinguish between a large early eluting endogenous peak extracted from urine and conjugate metabolites. Therefore, there was a possibility that conjugates were present in urine but were not identified. Urinary data are presented to corroborate serum data. When ADR–DONE and AOL–DONE were present in serum they were also present in urine and when they were not present in serum they were also not present in urine (Tables 2 and 3). AOL was detected in the urine of all patients studied.

DISCUSSION

A combination of three features distinguishes this study from previous reports of ADR pharmacokinetics: (a) a large group of patients was studied and blood sampling was frequent; (b) chemically pure reference compounds of several potential metabolites of ADR were synthesised as standards for both HPLC and TLC assays; and (c) major *in vivo* serum metabolites were isolated and identified by HPLC, TLC and mass spectrometry. The main findings of the study are summarised in four points.

1. Several metabolites of adriamycin were present in the serum of patients. Most were short-lived with the exception of two: adriamycinol and adriamycinol 7-deoxyaglycone.
2. Deoxyaglycone metabolites were present in only 60% of all patients.
3. Inter-patient variations in ADR metabolism were dictated by whether or not 7-deoxyaglycone metabolites were formed.
4. Variations in the AUC of ADR correlated with the relative amount of metabolism of the drug. Thus, biotransformation may also be considered a factor which can modulate the pharmacokinetics of ADR.

Aglycones of ADR have been the subject of a controversy in the past. They have been claimed to be both *in vivo* metabolites [14] and artefacts of analytical methodology [18]. The controversy appears to be between the techniques of TLC and HPLC. When biotransformation of ADR was first investigated in man only TLC was available.

Table 3. Inter-patient variations in the metabolism of adriamycin: 24 hr *cumulative excretion of metabolites in urine. Urinary data are presented to complement the serum data of Table 2. When ADR–DONE and AOL–DONE were detected in serum, they were also detected in urine. Patient WG‡ appears to be an exception, however, urinary excretion of* ADR *in this patient was low therefore levels of 7-deoxyaglycones could have been too low to be detected*

Patient	Dose (mg)	ADR (mg)	AOL (μg)	AOL–DONE (μg)	ADR–DONE (μg)	SERUM AGLYCONES AOL–DONE AUC†	SERUM AGLYCONES ADR–DONE AUC
JJ	75	0.5	360	310	170	76	3
MMcC	70	3.9	370	96	17	39	35
EB	100	5.9	330	ND*	ND	ND	ND
IC	75	3.6	1000	ND	ND	ND	ND
JH	62	1.3	180	410	1200	180	152
LMcG	57	3.1	200	ND	ND	ND	ND
‡WG	50	0.8	43	ND	ND	6	6
AB	30	1.9	130	ND	ND	ND	ND
MG	35	1.9	96	43	190	23	23

*Not detectable
†ng/ml × hr

These early studies suggested that ADR was extensively metabolised to aglycones. Five were isolated and identified in urine, six were detected in bile and up to three detected in plasma [14,19,20]. However, when new HPLC methods were introduced no evidence was found for aglycones being present in either urine, bile or plasma [8,18,21–23]. After Israel and co-workers demonstrated that ADR and AOL could be spontaneously converted into 7-hydroxyaglycones (ADR–ONE and AOL–ONE, Fig. 1) *in situ* on TLC plates, aglycones were subsequently challenged as being TLC artefacts [8,18,21]. Silica gel has acidic properties and acid catalysed hydrolysis of the glycosidic bond of ADR to produce a 7-hydroxyaglycone occurs under relatively mild conditions [13]. Indeed, Takanashi and Bachur [14], who identified five aglycones in human urine, conjectured that two 7-hydroxyaglycones could be chemical artefacts. Deoxyaglycones are unlikely chemical degradation products. They are formed by complex enzyme catalysed bioreductions and are not *in vitro* chemical decomposition products [15]. Thus, the two 7-deoxyaglycones identified in this study are most likely to be true aglycone metabolites of ADR. Recent improved HPLC techniques have now also detected 7-deoxyaglycone metabolites of ADR in man [15,24,25]. Previous conflicting data may be partly explained by inter-patient variations in biotransformation of ADR to 7-deoxyaglycones similar to the type we have described.

Other studies have suggested that there are marked inter-patient variations in conversion of ADR to aglycones. For example, an aglycone was detected (but not identified) in only a proportion of all the patients studied and because of this was explained by an irreproducible extraction technique [26]. The technique in question involved extraction of urine with the alcohol butan-1-ol. Aglycones are more soluble in alcohols than most common solvents including chloroform [15]. In another report where both ADR–DONE and AOL–DONE were identified in plasma by HPLC, the authors were unable to detect the aglycones in all patients studied [24]. Finally, Chan and co-workers, who detected two aglycones in plasma by TLC (which were not 7-hydroxyaglycones), noticed that they were also not present in every patient studied [27.28].

Limited data on the kinetics of ADR aglycones are available because of the problems of detection and identification. The kinetics of AOL are well characterised and similar to our findings [8,23]. Benjamin and co-workers [20], who detected three aglycones in plasma by TLC, showed that one of these exhibited kinetics with a qualitative inter-patient variation. In five patients peak plasma concentrations occurred 8 hr after drug administration. In seven patients peak plasma concentrations occurred during the first hour after drug administration. The aglycone was referred to as AAM and is similar in behaviour to the aglycone metabolite we have identified as adriamycinol 7-deoxyaglycone (AOL–DONE). Recently, plasma profiles of ADR–DONE and a combined AOL–DONE/ADR–ONE HPLC peak have been described in six patients [25]. Levels of ADR–DONE were low and the combined AOL–DONE/ADR–ONE peak, which was the major aglycone species detected, reached peak levels approx. 10 hr after drug administration. In that report, only two blood samples were taken during the first hour of treatment which could have precluded detection of early peak levels of aglycone metabolites.

The production of 7-deoxyaglycone metabolites of ADR has been linked to the evolution of reactive and free radical intermediates which can theoretically participate in and alter the anti-cancer activity and cardiotoxicity of the drug [29,30]. From this viewpoint 7-deoxyaglycones may be important indicators of pharmacological activity in man. *In vitro* studies with anaerobic rat liver microsomes have shown that 7-deoxyaglycones are formed by a linear sequential pathway [31,32]. ADR is first converted to ADR–DONE rapidly, which is then converted to AOL–DONE without going through AOL. A similar and equally rapid pathway of formation also occurs *in vivo* in both the heart and liver of mice [33]. In this case serum AOL–DONE followed closely its formation in tissues. The serum profiles of ADR–DONE and AOL–DONE in man, although complex and subject to marked inter-patient variations, are broadly consistent with the above pathway. ADR–DONE appeared quickly and was cleared quickly. AOL–DONE appeared later and was cleared less quickly. If serum levels of AOL–DONE reflect its production in the heart and other tissues in man, then high levels may also indicate a large production of toxic reactive intermediates. MB and JJ were the only patients studied that developed signs of ADR induced cardiotoxicity. These two patients also had high levels of circulating AOL–DONE. At this stage a correlation between metabolism and toxicity is only speculative. However, these data suggest an area for future studies to investigate the pharmacological significance of 7-deoxyaglycone metabolites of ADR in man.

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