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Hepatic extraction, metabolism and biliary excretion of doxorubicin in the isolated prefused rat liver

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Summary. The hepatic extraction, metabolism, and biliary excretion of doxorubicin (DX) were studied in the isolated perfused rat liver. Three doses of DX equivalent to 2, 20, and 100 mg/kg in rats were studied over a period of 3 h after bolus injection into the reservoir. DX and metabolites concentration in perfusate, bile, and liver were determined by high-pressure liquid chromatography.

The hepatic extraction ratio was low (<0.24) and decreased progressively over the 3 h. The hepatic extraction and clearance were significantly lower at the highest dose. Doxorubicinol (DX-OL) was the only metabolite detected in the perfusate, accounting for less than 4% of the total AUC. Thirty-one to thirty-three percent of the dose was excreted into bile over 3 h as unchanged DX. This was reduced to 22% at the highest dose. Only 0.35%–1.33% of the dose was excreted as DX-OL. DX aglycones were found only in the liver, where they represented 20%–30% of the total fluorescence at 3 h.

In conclusion, in this model DX has a low extraction ratio, is poorly metabolized and extensively excreted into bile.

Introduction

Doxorubicin (DX, adriamycin) is an anthracycline antibiotic widely used in cancer chemotherapy [17, 28]. DX is mainly eliminated by the liver and excreted into bile [5, 19]; thus hepatic clearance is a primary determinant of DX plasma concentration. However, although there have been many studies on DX pharmacokinetics [5, 21], there are very few data on the hepatic extraction and clearance of this drug. Furthermore, the specificity of the analytical methodology used in some previous studies has been questioned [26]. The aim of this work was therefore to study the hepatic extraction, metabolism, and biliary excretion of DX in the isolated perfused rat liver, using a highly sensitive and specific high-pressure liquid chromatography (HPLC) assay [20, 26]. This experimental model was used since it allows easy sampling of the liver portal inflow and venous outflow and thus direct estimation of hepatic extraction.

Materials and methods

Liver perfusion. Male Sprague-Dawley rats (Charles River Laboratories, France; wt 286±50 g) were used. The animals were fasted for 12 h before the experiment but were given free access to water with 50 g/l glucose. They were anesthetized with pentobarbital sodium (60 mg/kg i.p.) and the liver surgically removed by the standard technique [14]. The bile duct was cannulated with polyethylene tubing (ID 0.30 mm; Biotrol, Paris). 1000 IU heparin (Laboratories Leo, Paris) in 1 ml of 9 g/l saline were injected into the saphenous vein. The portal vein was then cannulated with a large polyethylene catheter (ID 2 mm, length 2 cm; Biotrol, Paris). The same type of cannula was used for all perfusions. The liver was immediately perfused with approximately 20 ml of the perfusion solution, excised, and transferred to a perfusion chamber. The entire procedure of liver removal took less than 10 min.

Livers (7.63±1.19 g) were perfused with a recirculating system essentially as described by Miller et al. [15]. The liver was perfused through the portal vein with Krebs-Ringer bicarbonate phosphate buffer containing rat whole blood (1/3 v/v, final hematocrit 15%), with 1000 IU/l heparin (Laboratories Leo, Paris) 30 g/l bovine serum albumin (Calbiochem, San Diego, Calif.), 1.5 g/l glucose, and 2.5 mmol/l Ca^{2+}. The volume of the perfusion medium was 180 ml.

The perfusate was oxygenated with a mixture of 95% O_2 and 5% CO_2 and the pH was kept close to 7.4. The perfusion was carried out at 37 °C in a thermostatically controlled cabinet using a recirculating system. The liver was perfused at a constant perfusion pressure of 13 cm of perfusate (9.5 mm Hg).

Experimental design. Over the initial 45–60 min, portal blood flow rose progressively; then a stable plateau was maintained. To compensate for the enterohepatic circulation of bile acids, sodium taurocholate (TC; Calbiochem, San Diego, Calif.) was perfused into the reservoir as loading dose of 6 μmol followed by 0.3 μmol min^{-1} in Krebs buffer. A tracer dose of sodium ^{14}C-TC (Amersham, England; specific activity 55.7 mCi/mmol) was added to the TC background infusion to a final specific activity of 20.8 μCi/mmol. This infusion rate was required to achieve a steady-state TC concentration of approximately 20 μ*M*. All experiments were started after 45–60 min of perfusion in order to achieve a maximal portal blood flow, and a steady state concentration of TC.

Doxorubicin hydrochloride (Adriblastine, Laboratories Roger Bellon, France) was injected as a bolus into the

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reservoir. To avoid photooxidative reaction of DX and fluorescent metabolites, the perfusion cabinet, syringes, and flasks containing the DX solution were thoroughly covered with aluminum foil. Serial samples were taken simultaneously from the portal inflow and the venous effluent 5, 10, 15, 20, 30, 60, 120, and 180 min after the injection. Bile was collected at 15-min intervals. Three doses of DX were studied: 0.27 mg, 2.7 mg, and 13.5 mg. With a perfusate of 180 ml and a blood-to-plasma partition coefficient of 1.7 (see below) these doses gave initial theoretical DX plasma concentrations of 0.882, 8.820, and 44.1 μg/ml respectively. These concentrations are approximately equivalent to those obtained in rat in vivo after i.v. injection of 2, 20, and 100 mg/kg respectively [1, 7, 11].

Two other series of experiments were performed: (1) bolus injection of 2.7 mg DX into the reservoir after 3 h perfusion without DX to test the stability of the perfused liver; (2) continuous perfusion of 2.7 mg of DX over 3 h (15 $\mu g.min^{-1}$) into the reservoir with the same sampling times as in the main experiments. Total loss of perfusate due to sampling was approximatively 15% of the total perfusate. At the end of each experiment, the liver was blotted dry and weighed. A fragment was fixed in Bouin solution and another immediately frozen in liquid nitrogen for DX determination in the liver. The microscopic morphology of the liver was studied after hematoxylin-eosin and trichrome staining.

Assays. DX concentrations in perfusate were determined by a specific and sensitive HPLC method which has been described previously [20]. Briefly, DX and metabolites were extracted by rapid purification on cartridges containing bonded silica (C_{18}-Sep-pak, Waters, Milford, Mass., USA) and were eluted from these minichromatographic columns with methanol. After evaporation of the solvent under nitrogen at 40 °C, the residue was dissolved in the mobile phase of the reverse-phase HPLC system. HPLC was performed on a Waters apparatus, using a column of micro-Bondapak-phenyl (Waters) and an isocratic eluting mixture of 0.1% formate buffer (pH 4) and acetonitrile (68/32, v/v). Detection was performed with a Schoefel model SF 970 fluorometer. Quantitation was achieved using daunorubicin as an internal standard. DX and metabolites were identified by comparison with authentic standards kindly supplied by Farmitalia (Milan). DX concentration was determined in bile according to the same technique, except that the extraction step was omitted. The bile sample was diluted in 0.05 *M* phosphate buffer, pH 7, and directly injected into the column. For DX determination in the liver, the liver was homogenized and extraction was performed according to Baurain et al. [4].

The concentration of TC in the perfusate was determined by measuring ^{14}C radioactivity. No account was taken of he presence of endogenous TC which represents a small fraction (<3%) of the total taurocholate in the system. Liquid scintillation counting (SL 32, Intertechnique, France) was carried out using automatic external standardization, counting 1 ml plasma in 5 ml Aqualite (JT Baker Chemicals, Deventer, Holland).

Aspartate aminotransferase (ALAT) activity was measured according to a standard technique and oxygen consumption was determined with a Lex-O_2-Con oxygen content analycer (Lexington Instruments, Waltham, Mass., USA).

Calculations. The biliary secretion was measured by weighing. Portal blood flow was determined directly by measuring the volume of hepatic venous effluent collected over 1 min.

Assuming that all DX injected into the perfusion is eliminated via the liver [i.e., if we neglect DX metabolism in RBC (see below) and nonspecific binding on tubing], the hepatic extraction ratio and clearance of DX can be calculated in two ways:

1. The blood hepatic clearance of DX (Cl_B) may be calculated as:

$$Cl_B = \frac{Dose}{R_B \times AUC_P},$$

where R_B is the blood-to-plasma concentration ratio (see below) and AUC_P the area under plasma concentration-time curve. AUC_P was calculated based on the trapezoidal rule. The mean estimated hepatic extraction ratio of DX (E_E) was then calculated as:

$$E_E = \frac{Cl_B}{Q_B},$$

where Q_B is the portal blood flow.

2. The hepatic extraction ratio (E) was also directly calculated from the plasma concentration of DX measured in the hepatic portal inflow (Ci) and in the hepatic venous outflow (Co) as:

$$E = \frac{Ci-Co}{Ci}.$$

The amount of DX excreted into bile was calculated from the bile flow and from the biliary concentration of DX.

The amount of DX and metabolites in the liver was calculated by multiplying DX and metabolite concentrations in the liver by the liver weight. To estimate liver function during the experiment, the intrinsic clearance of TC (Cl_{int} TC) was calculated according to the "sinusoidal" perfusion model of hepatic elimination [3, 27] as:

$$Cl_{int}\,TC = -\,Q \ln (1-E_{TC}),$$

where Q is the liver plasma flow and E_{TC} the hepatic extraction ratio of TC calculated from the steady state concentration of TC in the portal inflow and the venous outflow. Cl_{int} can be defined as the maximal clearance of substrates by the liver in the absence of any flow limitation. Under first-order conditions Cl_{int} is therefore a flow-independent parameter which can be used as a quantitative index of functional hepatic mass [25].

Statistics. Data are presented as mean ± SD unless specified. Analysis of data were made using the Student's *t* test for paried and unpaired comparison and analysis of variance.

Blood to plasma concentration ratio. The distribution and metabolism of DX in rat red blood cells was studied in experiments where the perfusate was recirculated without liver during 4 h. The blood concentration of DX (C_b) was calculated as:

$$C_b = C\,(1\text{-}Ht) + C_{bc} \cdot Ht$$

where C is the plasma concentration of DX, C_{bc} the concentration of DX in RBC, and Ht the hematocrit [22]. The

blood plasma concentration ratio (R_b) was then calculated as:

$$R_b = \frac{C_b}{C}.$$

In this study, the value of 1.70 ± 0.32 was obtained for R_b, which did not vary significantly over the range of concentrations studied. A small fraction of DX was metabolized in RBC to doxorubicinol (DX-OL). The calculated fractional rate of DX-OL formation in RBC was 0.034 h^{-1} and the metabolic clearance of DX by RBC was estimated to 0.15 ml · min^{-1}. This represented less than 4% of the total blood clearance for all doses of DX studied and therefore was neglected in the calculation.

Results

No significant variation in portal blood flow was observed after DX injection over the experimental period.

Hepatic clearance and estimated extraction of DX calculated from the AUC_P for the different doses studied (Fig. 1) are indicated in Table 1. The estimated hepatic extraction ratio ranged from 0.13 to 0.24; hepatic clearance and estimated extraction were similar at the two lower doses, but decreased significantly at the higher dose. The hepatic extraction ratio calculated from DX concentration in the portal inflow and the venous effluent was in the range of that estimated from the area under the curve during the 1st h (Table 2). It was also significantly reduced at the higher dose. However, there was a progressive significant decrease of the extraction ratio over the 2nd and 3rd h. Doxorubicinol (DX-OL) was the only metabolite detected in the perfusate. DX-OL AUC_P accounted fpr 3.96% and 3.34% of the total AUC_P in the perfusate at the doses of 2.7 and 13.5 mg respectively. DX-OL concentration at the lowest dose (0.27 mg) was too low to allow precise estimation of the AUC_P. No aglycones were detected in the perfusate.

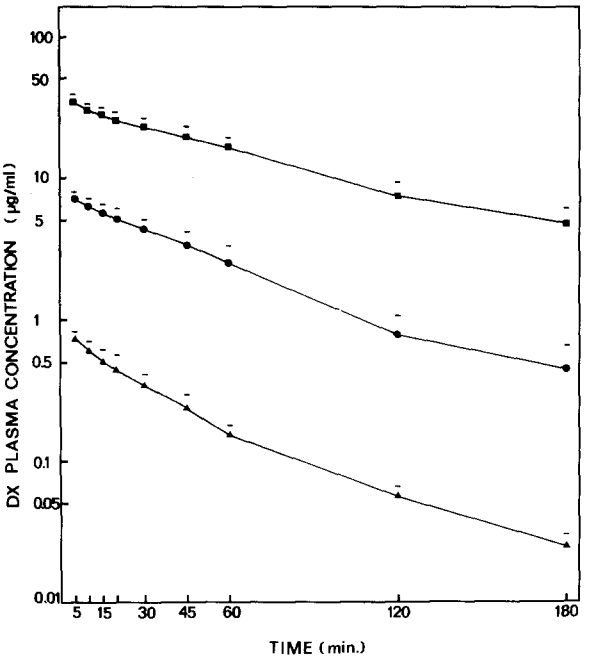

Fig. 1. Plasma doxorubicin *(DX)* concentration-time curves after injection of three different doses into the reservoir of the isolated rat liver perfusion system. ▲ 0.27 mg; ● 2.7 mg; ■ 13.5 mg

DX was detected in bile in the first 5 min after injection. The excretion rate was maximal at 60 min. The cumulative amount of DX excreted into bile for the different doses studied is indicated in Table 3. Thirty-one to thirty-three percent of the injected dose was exereted into bile 3 h

Table 1. Hepatic clearance (Cl_B) and estimated extraction of DX (E_E) after bolus injection into the reservoir (mean ± SD)

DX dose (mg)	*n*	AUC_P (µg · ml^{-1} · min^{-1})	Cl_B (ml · min^{-1} · g^{-1} liver)	Q_B (ml · min^{-1} · g^{-1} liver)	E_E
0.27	6	31.9± 5.5	0.600±0.124	2.64±0.81	0.239±0.07
2.70	6	458.0± 92.3	0.553±0.080	2.43±0.65	0.236±0.04
13.50	6	3018.0±487.0	0.376±0.064*	2.87±0.46	0.130±0.005*

AUC_P, area under plasma concentration-time curve; Q_B, portal blood flow
* $p < 0.01$ vs DX dose 0.27 and 2.7 mg

Table 2. Hepatic extraction of DX (mean ± SD) over the experimental period after bolus injection or during infusion

DX dose (mg)	*n*	Time (min)				
		10	30	60	120	180
Bolus injection						
0.27	6	0.271±0.05	0.246±0.012	0.238±0.060	0.141±0.021	0.044±0.023
2.70	6	0.216±0.039	0.289±0.070	0.271±0.026	0.189±0.400	0.067±0.037
13.50	6	0.173±0.005*	0.208±0.044	0.156±0.036**	0.089±0.021**	0.042±0.028
Perfusion (3 h)						
2.70	5	0.181±0.03	0.164±0.078	0.139±0.034	0.140±0.077	0.195±0.025

There is a significant ($p < 0.05$) decrease at 120 and 180 min for all bolus doses
* $p < 0.05$ vs dose 0.27 mg; ** $p < 0.01$ vs dose 2.7 mg (see text for details)

Table 3. Cumulative amount of DX excreted into bile over the experimental period (% of injected dose; mean ± SD)

DX dose (mg)	*n*	Time (min)		
		60	120	180
0.27	6	10.10±3.9	23.2±5.6	32.8±7.4
2.70	6	9.90±2.2	22.3±2.8	30.7±4.1
13.50	6	7.15±2.3	17.1±5.9	22.1±7.5*

* $p < 0.05$ vs doses 0.27 and 2.7 mg

after injection as unchanged DX. This was reduced to 22% with the highest dose (Table 3). Only 0.35%–1.33% of the dose was excreted into bile as DX-OL over 3 h. No aglycones were detected in bile.

Ten min after DX injection the intrinsic clearance of TC was not significantly different from basal values (Tables 4, 5). However, there was a significant decrease in biliary flow and intrinsic clearance of TC over the 2nd and 3rd h. This was observed with all doses but was significantly more pronounced at 180 min with 13.5 mg. For control livers, there was no significant change in intrinsic clear-

Table 4. Biliary flow (μl · h^{-1} · g^{-1} liver; (mean ± SD) before and after DX injection, during perfusion, and in controls

DX dose (mg)	*n*	Time (min)			
		0	60	120	180
Bolus injection					
0.27	6	108.5± 6.9	93.7± 4.1	77.40± 5.3	57.8±11.6
2.70	6	107.9±18.1	101.1±13.7	86.60±13.7	67.2±19.1
13.50	6	125.3±21.1	108.1± 7.4	92.73± 2.7	65.8± 9.0
3-h perfusion					
2.7	6	115.0±25.3	106.3±21.0	85.60±16.1	84.5±15.2
Controls					
	6	110.2±15.2	105.1±10.1	92.30± 7.2	81.1± 9.1

There is a significant decrease at 2 and 3 h for all bolus doses ($p < 0.05$) and at 2 h for the perfusion vs values at 1, 2, and 1 h respectively. There is a significant decrease at 3 h for the bolus doses vs controls ($p < 0.05$)

Table 5. Intrinsic clearance of taurocholate (ml · min^{-1} · g^{-1} liver; mean ± SD) after DC injection, during DX infusion, and in controls

DX dose (mg)	*n*	Time (min)				
		0	10	60	120	180
Bolus injection						
0.27	7	6.80±0.22	6.94±0.32	7.45±1.01	5.98±1.38	5.11±1.21
2.70	6	6.01±1.30	6.14±1.13	7.53±1.04	6.09±1.20	5.24±1.02
13.50	7	6.60±0.30	7.60±1.42	8.14±1.99	5.05±1.40*	3.20±1.40*
Perfusion						
2.70	5	7.52±0.45	7.52±0.38	6.67±0.55	6.28±0.60	5.26±0.72
Controls						
	6	7.00±0.20	7.01±0.10	6.92±0.50	6.87±0.30	5.75±0.40

* $p < 0.05$ vs 0.27 and 2.7 mg
There is a significant ($p < 0.05$) decrease at 120 and 180 min for all bolus doses and at 180 min for the infused dose vs values at 60, 120, and 180 min respectively and vs controls

Table 6. DX and metabolites recovered in the liver 3 h after injection (mean ± SD)

DX dose (mg)	*n*	Total anthracyclines recovered in liver (% of dose injected)	Percentage of total fluorescence			
			DX	DX-OL	7-deo-DX	7-deo-DX-OL
0.27	6	46.40±6.1	74.44±2.43	4.33±0.016**	11.08±2.35	10.15±2.00
2.70	6	44.99±5.1	71.20±5.72	2.36±0.220	16.44±4.40	9.99±1.92
13.50	6	36.34±4.1*	66.67±0.71	2.70±0.640	24.08±1.68***	6.50±1.39

DX, doxorubicin; DX-OL, doxorubicinol; 7-deo-DX, 7-deoxy-doxorubicin; 7-deo-DX-OL, 7-deoxy-doxorubicinol
* $p < 0.01$ vs DX dose 0.27 and 2.7 mg; ** $p < 0.05$ vs DX dose 2.7 mg; *** $p < 0.01$ vs DX dose 0.27 mg

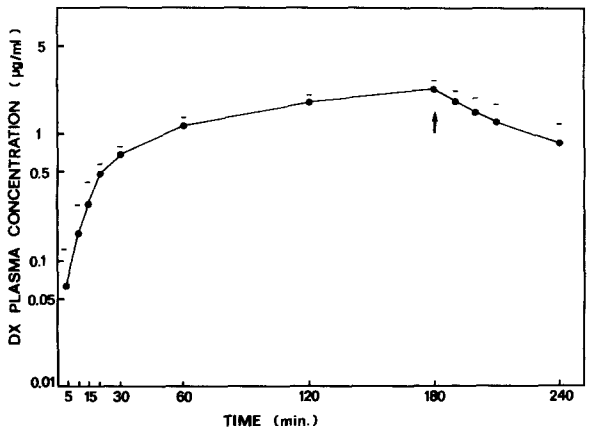

Fig. 2. Plasma doxorubicin *(DX)* concentration-time curve during a 3-h infusion (15 μg/min) into the reservoir of the isolated rat liver perfusion system. *Arrow*, end of the infusion

ance of TC and a slight decrease in biliary flow which became significant compared with basal values only during the 3rd h.

The amount of DX and metabolites recovered in the liver at 180 min is indicated in Table 6. There was a significant reduction of the fraction of the injected dose recovered in the liver at the higest dose. DX aglycones (7-deoxyDX and 7-deoxyDX-OL) represented 21%–31% of the total fluorescence recovered in the liver.

When 2.7 mg DX was infused into the reservoir over 3 h (15 μg · min^{-1}), steady-state plasma concentrations of DX were not achieved (Fig. 2). Hepatic extraction was significantly lower during the 1st h compared with that measured after bolus injection of the lower doses. (Table 2) However, in contrast to bolus injection, there was no significant variation of hepatic extraction over the 3 h. The biliary flow was significantly higher during the 3rd h than after bolus injection, in spite of a similar decrease in intrinsic clearance of TC. When 2.7 mg DX were injected into the reservoir after 3 h of perfusion without DX, the elimination half-life and extraction of DX over 1 h were not significantly different from that in the standard protocol.

ALAT activity and O_2 consumption were in the normal range and did not vary significantly over the experimental period.

Discussion

In the present study, the hepatic elimination of DX was studied in the isolated perfused rat liver. A main finding was that DX was, in this model, a drug with a low extraction ratio, extensively secreted into bile and poorly metabolized.

The estimated hepatic extraction ratio calculated from the DX AUC_P and the liver blood flow ranged from 0.13 at the higher dose to 0.24 at the lower doses and was in accordance with the hepatic extraction ratio calculated directly from DX concentration in the portal vein and the venous effluent.

These values were obtained with livers perfused at a flow rate ranging from 2.43 to 2.87 ml · min^{-1} · g^{-1} liver, a liver blood flow value higher than that usually reported in the rat in vivo (1.5–2 ml · min^{-1} · g^{-1} liver [25]. Livers were perfused at a constant pressure of 9.5 mmHg, which is in the range of physiological pressure in vivo and ensures an homogeneous perfusion of the liver; but since the viscosity of the perfusion medium used was lower than that of normal blood, a high flow value was obtained in our preparation. Since extraction varies inversely with the flow rate [25], the measured hepatic extraction ratio should be slightly higher at a lower flow rate but should not exceed 0.30–0.35 [25]. Therefore, in our model DX is a compound with a low hepatic extraction.

There are very few data on the hepatic extraction of DX [2, 9, 10]. In the rabbit [10] the bioavailability of DX was estimated to be 0.6. The hepatic extraction of DX was determined in five patients with liver metastasis and was estimated to range from 0.05 to 0.5 [9]. In patients with cirrhosis and hepatocellular carcinoma we found an extraction ratio ranging from 0 to 0.10 [2]. Further studies are needed in patients with normal liver function since the finding that DX is a poorly extracted drug would have two major implications: (1) Its clearance would depend mainly on the hepatic intrinsic clearance and the unbound fraction of the drug in the plasma, and would be relatively independent of liver blood flow [25]. One might, therefore anticipate important modifications in DX pharmacokinetics when the liver function is impaired. (2) The advantage of DX administration via the hepatic artery over i.v. infusion would appear very low, since it has been shown that for drugs eliminated only in the perfused region, the therapeutic advantage for drug delivery is inversely related to the extraction ratio [8].

In our study, the hepatic extraction of DX was relatively stable during the 1st h after DX injection but decreased progressively over the 2nd and the 3rd h. This could be secondary to a progressive loss of the viability of the perfused liver; however, DX elimination half-life and extraction during the 1st h was unchanged when DX was injected after 3 h of perfusion without DX. Furthermore, liver function assessed by the determination of the intrinsic clearance of TC was stable over the experimental period in control livers.

The diminution of extraction could be secondary to a toxic effect of DX on the liver, impairing biliary flow. Indeed, Tavolini and Guarino have shown that biliary flow is a critical determinant of DX biliary excretion in the rat [24]. Although the microscopie morphology of the liver was unchanged at the end of the experiments and ALAT activity in the venous effluent did not rise significantly, there was a slight but significant decrease of biliary flow and intrinsic clearance of TC over the last 2 h and this reduction was more pronounced with the higher dose. This delayed effects of DX on biliary secretion is not in agreement with the work of Tavolini et al., who observed no significant effect of DX on biliary flow over 6 h in rats in vivo [24]. Whether this discrepancy may be explained by different experimental conditions is unclear. However, when DX was perfused into the reservoir over 3 h, the hepatic extraction ratio remained stable over the whole period in spite of a significant decrease in biliary flow during the 2nd h and a parallel decrease in intrinsic clearance of TC. Therefore, it is difficult to decide whether the progressive diminution of DX hepatic extraction ratio observed after bolus administration is a toxic effect of DX on its own transport via a reduction in biliary flow or a "true" result of nonlinear kinetics in protein binding or tissue distribution. Similarly, it is difficult to know whether the

dose-dependent elimination of DX, attested by reductions in hepatic clearance, hepatic extraction, and the fraction of the dose excreted into bile at the highest dose, is a dose-dependent toxic effect of DX or a result of "true" saturation kinetics [6, 18]. Whatever the precise mechanism, this was observed at the highest dose, which is far above those commonly used in standard protocols. Indeed, this dose of 13.5 mg in our study gave DX plasma concentrations ranging from 35 to 5 μg/ml; this is approximately 10 times higher than the plasma concentration commonly observed at 10 mg/kg in the rat in vivo [7], a dose which is roughly comparable to 60 mg/m^2 in man.

In our study, DX was extensively excreted into bile without prior metabolism since approximately 30% of the initial dose appeared in the bile over a 3-h period as unchanged DX and less than 2% as DX-OL. Neither aglycones nor conjugates were found in the bile. This is in agreement with the work of Tavolini [24] and Peters [16] in rats in vivo, confirming that DX is more extensively excreted into bile than previously thought [1, 7, 11, 12, 23]. This is not surprising in view of its high molecular weight and its amphipathic properties [13]. Furthermore, using a very sensitive and specific HPLC assay, our study indicates clearly that DX is poorly metabolized in the rat. Indeed, DX-OL was the only metabolite detected in the perfusate and in the bile, accounting for 4% and 1% of the total fluorescence respectively. Aglycones were found only in the liver, where they represented 20%–30% of the total fluorescence at 3 h. The significance of these aglycones is unclear, since in parallel experiments performed in rats in vivo, using the same analytical methodology, we were unable to find aglycones in the liver 3 h after i.v. injection of DX (J. Robert, unpublished data). It is well known that aglycones may be generated in anaerobic conditions [17]; however, O_2 consumption values of the isolated perfused livers were in the normal range and did not decrease significantly over the experimental period. It must be stressed that these aglycones do not leave the liver, since they were found neither in the perfusate nor in the bile. As emphasized previously [26], the number of metabolites reported previously are probably artifactual and related to the analytical methodology.

References

1. Arcamone F, Lazzati M, Vicario GP, Zini G (1984) Disposition of ^{14}C-labelled 4'-epidoxorubicin and doxorubicin in the rat. Cancer Chemother Pharmacol 12: 157
2. Ballet F, Barbare JC, Poupon R (1984) Hepatic extraction of adriamycin in patients with hepatocellular carcinoma. Eur J Cancer Clin Onc 20: 761
3. Bass L, Keiding S, Winkler K, Tygstrup N (1976) Enzymatic elimination of substrates flowing through the intact liver. J Theor Biol 61: 393
4. Baurain R, Zenebergh A, Trouet A (1978) Cellular uptake and metabolism of daunorubicin as determined by high-pressure liquid chromatography: application to L1210 cells. J Chromatogr 157: 331
5. Benjamin RS, Riggs CE, Bachur NR (1973) Pharmacokinetics and metabolism of adriamycin in man. Clin Pharmacol Ther 14: 592
6. Boston RC, Philips DR (1983) Evidence of possible dose-dependent doxorubicin plasma kinetics in man. Cancer Treat Rep 67: 63
7. Celio LA, DiGregorio GJ, Ruch E, Pace JN, Piraino AJ (1982) Doxorubicin concentration in rat plasma, parotid saliva, and bile and protein binding in rat plasma. Arch Int Pharmacodyn 260: 180
8. Collins JM, Dedrick RL (1982) Pharmacokinetics of anticancer drugs. In: Chabner B (ed) Pharmacological principles of cancer treatment, Philadelphia, WB Saunders, pp 77–100
9. Garnick MB, Ensminger WD, Israel MA (1979) Clinical pharmacological evaluation of hepatic arterial infusion of adriamycin. Cancer Res 39: 4105
10. Harris PA, Gross JF (1975) Preliminary pharmacokinetic model for adriamycin. Cancer Chemother Rep 59: 819
11. Hartman N, Basseches PJ, Powis G (1982) Effect of cyclophosphamide pretreatment on the short-term disposition and biliary excretion of adriamycin metabolites in rats. Cancer Chemother Pharmacol 10: 11
12. Israel M, Wilkinson PM, Pegg WJ, Frei E (1978) Hepatobiliary metabolism and excretion of adriamycin and *N*-trifluoroacetyladriamycin-14-valerate in the rat. Cancer Res 38: 365
13. Klaassen CD, Watkins JB (1984) Mechanisms of bile formation, hepatic uptake and biliary excretion. Pharmacol Rev 36: 1
14. Miller LL (1973) Technique of isolated perfused rat liver. In: Bartosek I, Guaitani A, Miller LL (eds) Isolated liver perfusion and its applications. Raven, New York, p 11
15. Miller LL, Bly LG, Watson ML, Bale WF (1951) The dominant role of the liver in plasma protein synthesis. A direct study of the isolated perfused rat liver with the aid of lysin. ξ–C^{14}. J Exp Med 94: 431
16. Peters JH, Gordon GR, Kashiwase D, Acton EM, Hunt CA (1985) Metabolic disposition of N,N-dibenzyldaunorubicin in the rat. Cancer Res 43: 1477
17. Myers CE (1982) Antracyclines. In: Chabner B (eds) Pharmacological principles of cancer treatment. WB Saunders, Philadelphia, pp 426
18. Powis G, Ames MM, Kovach JS (1981) Dose-dependent pharmacokinetics and cancer chemotherapy. Cancer Chemother Pharmacol 6: 1
19. Riggs CE, Benjamin RS, Serpick AA, Bachur NR (1977) Biliary disposition of adriamycin. Clin Pharm Ther 22: 234
20. Robert J (1980) Extraction of anthracyclines from biological fluids for HPLC evaluation. J Liq Chromatogr 3: 1561
21. Robert J, Illiadis A, Hoerni B, Cano JP, Durand M, Lagarde C (1982) Pharmacokinetics of adriamycin in patients with breast cancer: correlation between pharmacokinetics parameters and clinical short-term response. Eur J Cancer Clin Oncol 18: 739
22. Rowland M, Tozer TN (1980) Clinical pharmacokinetics, Philadelphia, Lea and Febiger, p 287
23. Skibba JL, Jones FE, ConDon RE (1982) Altered hepatic disposition of doxorubicin in the perfused rat liver at hyperthermic temperatures. Cancer Treat Rep 66: 1357
24. Tavolini N, Guarino AM (1980) Bile secretory function: a determinant of adriamycin disposition. Arch Int Pharmacodyn Ther 245: 180
25. Wilkinson GR, Shand DG (1975) A physiological approach to hepatic drug clearance. Clin Pharmacol Ther 18: 377
26. Wilkinson PM, Israel M, Pegg WJ, Frei E (1979) Comparative metabolism and excretion of adriamycin in man, monkey and rat. Cancer Chemother Pharmacol 2: 121
27. Winkler K, Bass L, Keiding S, Tygstrup N (1979) The physiological basis for clearance measurements in hepatology. Scand J Gastroent 14: 439
28. Young RC, Ozols RF, Myers CE (1981) The anthracycline antineoplastic drugs. N Engl J Med 305: 319.

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