Cancer Chemother Pharmacol (1998) 41: 370–376

ORIGINAL ARTICLE

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Investigation of the enterohepatic recirculation of Adriamycin and its metabolites by a linked-rat model

Received: 21 January 1997 / Accepted: 14 July 1997

Abstract We investigated the possible role of enterohepatic recirculation in prolongation of the half-life of elimination for Adriamycin, a commonly prescribed anticancer agent. We sought to determine whether enterohepatic recirculation of Adriamycin and its metabolites occurs using a linked-rat model. Two rats, a donor and a receiver, were linked via a catheter from the bile duct of the donor rat to the duodenum of the receiver. Control experiments were conducted with intact rats (without a bile duct cannula, control A) in order to estimate the half-life of elimination and with bile duct-cannulated rats (control B) to determine the amounts of Adriamycin and its metabolites in the bile. [^{14}C-14]-Adriamycin was injected intravenously via the femoral vein to control A, control B and donor rats. The biological half-life of Adriamycin in the intact rats (control A, 10 h) was significantly higher than in the bile-duct-cannulated rats (control B, 4 h). The cumulative amount of Adriamycin and its metabolites excreted in the urine of the control A rats was also greater than from control B rats, indicating higher levels of the drug in their systemic circulation. Biological samples (bile, urine, plasma, blood cells and the major organs heart, liver and kidney) of the receivers contained significant amounts of Adriamycin and its metabolites. The total radioactivity recovered in the bile of the receivers accounted for 0.1% to 8% of the Adriamycin dose that was administered to the donors. Adriamycin and its metabolites appeared there only after a lag time that was consistent among all the receivers. Doxorubicinol aglycone was the major metabolite found in the bile and urine of the receivers. Low but constant levels of radioactivity were also detected in the plasma and blood cells of the receivers. The presence of unchanged Adriamycin in the bile and urine of the receivers suggested absorption of the parent drug from the intestine of the receivers. Overall, we estimated that about 22% of the dose injected to the donors was absorbed from the intestine of the receivers. Taken together, these findings clearly demonstrate a significant role for enterohepatic recirculation of Adriamycin and its metabolites, which may contribute to the ability of these compounds to induce cumulative cardiac damage and/or to increase the efficacy of Adriamycin.

Key words Enterohepatic recirculation · Adriamycin

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Introduction

Adriamycin (doxorubicin, ADR) is an anthracycline antibiotic with a wide spectrum of antitumor activity and a long elimination half-life. The successful use of Adriamycin as an antitumor agent, however, has been hindered by its well-described, but poorly understood, cardiac toxicity [1–4]. Several studies have shown that Adriamycin has a long but variable elimination half-life in both humans and animals [5–7]. For example, in patients receiving Adriamycin (50 mg/m^2) for treatment of breast cancer, the terminal half-life of the plasma concentration-time curve has been reported to be 70 h [8]. In a different study, in patients who received 60 mg/m^2 of Adriamycin, the terminal half-life has been shown to be 48.4 h [9]. The plasma concentration-time curve of Adriamycin can be described by a biexponential model, which is characterized by a distribution half-life of 5 to 10 min and a terminal half-life of 26 ± 17 h in humans [10].

Adriamycin is eliminated primarily in the bile and feces [11]. It appears in the bile within 3–5 min of an i.v. bolus injection of the drug [12, 13]. Unchanged Adriamycin is the major form of the drug that is excreted in

the bile and urine, followed by smaller amounts of the alcohol metabolite, doxorubicinol, and aglycones [9]. In experiments in rats with cannulated bile duct and bladder, 33–35% of the injected doses of 5, 20, or 40 mg/kg Adriamycin was excreted in the bile during a 10-h collection period, whereas only 4–8% was eliminated in the urine [14].

We hypothesized, based on its long elimination half-life and extensive biliary excretion (mostly in its unchanged form), that Adriamycin undergoes enterohepatic recirculation, and that this may play a role in the variability of the biological half-life of Adriamycin, and hence lengthen the exposure of sensitive organs to Adriamycin and/or its metabolites. In this study we used a linked-rat model, in which the bile-duct of donor rats, injected with radiolabeled Adriamycin via their femoral vein, was linked to the duodenum of receiver rats, to determine whether Adriamycin and its metabolites undergo enterohepatic recirculation within the receiver rats.

Material and methods

Materials

Male CD rats (250–300 g) were purchased from Taconic Farms (Germantown, N.Y.). Radiolabeled [^{14}C-14] Adriamycin with specific activity 100 μCi/mg was purchased from Amersham Corp. (Arlington Heights, Ill.). Nonradiolabeled Adriamycin (lyophilized powder) was provided by Adria laboratories (Dublin, Ohio) and its major metabolites, doxorubicinol (DOXOL), doxorubicin aglycone and doxorubicinol aglycone were provided by Farmitalia (Milano, Italy). HPLC grade solvents, polyethylene tubing (PE-10), liquid scintillation cocktail (Scintiverse E) and hydrogen peroxide (30%) were purchased from Fisher Scientific (Springfield, N.J.). Tissue solubilizer (BTS-450, Beckman Tissue-Solubilizer-450) was purchased from Beckman Instruments Inc. (Fullerton, Calif.). All other chemicals were of analytical grade.

Experimental protocol

Surgical procedure

Animals were randomly assigned into four groups: control A, control B, donors and receivers (five or six animals per group). Animals were anesthetized by i.p. injection of sodium pentobarbital and kept under anesthesia throughout the experiment (50 mg/kg, 12 h). The bile duct of control B animals, donors and receivers was cannulated using a polyethylene catheter (PE 10). Following isolation of the bile duct by removing the surrounding connective tissues, a midline incision was made in the duct and a polyethylene catheter (PE 10) inserted toward the liver (opposite to the direction of bile flow). The catheter was secured in place and sealed by making a node around the duct and the catheter using a silk suture. Pairs of rats (donors and receivers) were linked together by inserting the bile duct catheter from a donor into the duodenum of a receiver. The inserted catheter was secured and sealed in the duodenum of the receivers by applying small droplets of tissue adhesive (Vetbond, 3M Corp., St. Paul, Minn.) around the catheter.

The tail vein of all animals was cannulated for blood sampling using a polyethylene catheter (PE 10) and a 20-gauge needle as trocar.

Dosing and sample collection

[^{14}C-14]-Adriamycin (specific activity 0.2 μCi/mg) was prepared and administered as a bolus injection (10 mg/kg) to control A rats, control B rats and donors via the femoral vein. Following the injection of the dose, serial blood samples (100 μl) were collected from the tail vein of control A rats, control B rats and donors at 5, 10, and 20 min, and 1 through 12 h every hour into heparinized tubes. Blood sampling from the receivers started 1 h after dosing the donors and continued up to 12 h.

Blood samples were centrifuged at 20 000 rpm (5 min) immediately following collection to separate the plasma. The separated plasma and blood cells were frozen in liquid nitrogen and kept at −20 °C until analyzed. Blood cells were subjected to tissue solubilization and decolorization with hydrogen peroxide (30%, 200 μl) prior to analysis. The total radioactivity in each fraction was determined using a liquid scintillation counter (LSC).

Bile samples (final volume 100–200 μl) were collected periodically into Eppendorf microcentrifuge tubes from receivers and control B animals. Urine samples were collected directly from the bladder of all animals at the end of the 12-h experiment. Bile and urine samples were immediately frozen in liquid nitrogen following collection and kept at −20 °C until analyzed.

Major organs (heart, liver and kidneys) were collected from donors, receivers and control B animals, at the end of the experiment. They were rinsed with ice-cold normal saline, blot dried, frozen in liquid nitrogen and stored at −20 °C in petri dishes until analyzed. The total radioactivity of the organs was measured by LSC following tissue solubilization and hydrogen peroxide (30%, 200 μl) treatment.

The concentrations of Adriamycin and its major metabolites in the urine and bile samples were measured by HPLC. The HPLC system consisted of an autosampler (Hitachi AS-2000), Waters chromatography pumps, C18 reversed phase RCM cartridge, Sentry C18 Novapak guard column and the RCM unit, combined with a Waters data module.The mobile phase consisted of methanol/ammonium formate buffer (0.1%, pH 4.0) at a ratio of 70:30. The flow rate was 2 ml/min. The online detectors were a fluorometer (excitation wavelength 480 nm and emission wavelength 540 nm) and radioactivity detector (Beta Flo, Packard). The retention times of Adriamycin and its major metabolites, doxorubicinol, doxorubicin aglycone and doxorubicinol aglycone, were confirmed using authentic standards.

The total radioactivity of the bile and urine samples is reported as cumulative amounts using the specific activity of the administered dose and the efficiency of the LSC detectors.

Data analysis

RSTRIP software (Version 4.0, MicroMath Scientific Software, Salt Lake City, Utah) was used for calculation of the initial estimates of the parameters and constants of the model and PCNONLIN software (SCI Software, Version 3.0, ClinTrial, Lexington, K.y.) was used for all pharmacokinetic analyses. DELTAGRAPH software was used for plotting the graphs.

The plasma data from each animal were individually fitted to the following two-compartment open model and the average of the parameters and constants with standard deviations were used in subsequent calculations.

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

Where Cp is the concentration in plasma, A and B are the coefficients of each exponential term, α and β are the first-order hybrid rate constants associated with distribution and elimination.

Under the assumptions that (1) the fraction of the dose excreted in the bile of control B animals was the same as that of donors and (2) the fractions of the dose eliminated as individual metabolites in the bile were also identical for both control B animals and donors, the following equations were used (the subscripts 'u' and 'b' denote the urinary and biliary parameter or constant, respectively.):

$$f_u = \frac{A_u}{\text{Dose}} \quad (2)$$

$$f_b = \frac{A_b}{\text{Dose}} \quad (3)$$

Table 1 Summary of pharmacokinetic parameters and constants for plasma concentrations of Adriamycin in control A and control B animals and donors according to the two-compartment model using PCNONLIN. Values are means ±SD ($n = 5$ for control A and B animals, and $n = 6$ for donors)

Parameter	Control A	Control B	Donors
$(T1/2)_\alpha$ (min^{-1})	53.30 ± 4.10	4.38 ± 0.83	5.63 ± 0.91
$(T1/2)_\beta$ (min^{-1})	630.0 ± 57.27*	223.5 ± 36.04	203.82 ± 47.95
AUC (μg · min/ml)	2483.4 ± 141.38*	879.8 ± 20.91	615.16 ± 87.61
TBC (ml/min)	1.06 ± 0.09*	3.78 ± 1.40	4.23 ± 1.08

*$P < 0.05$

$$(fm)_b = \frac{(Am)_b}{Dose} \quad (4)$$

$$(fm)_u = \frac{(Am)_u}{Dose} \quad (5)$$

Where f_u and f_b are the fractions of dose excreted unchanged, A_u and A_b are the cumulative amounts of unchanged Adriamycin, $(Am)_u$ and $(Am)_b$ are the cumulative amounts of individual metabolites and $(fm)_u$ and $(fm)_b$ are the fractions of individual metabolites.

The biliary clearance (Cl_b) of Adriamycin and its metabolites $(Cl_b)_m$, were estimated by using total body clearance of control B animals (Cl_T) and the following equations:

$$Cl_b = f_b * Cl_T \quad (6)$$

$$(Cl_b)_m = (fm)_b * Cl_b \quad (7)$$

The following equation was used to calculate the total fraction of dose converted to individual metabolites:

$$(fm)_u + (fm)_b = fm \quad (8)$$

The total amount of unchanged drug at the intestinal site of absorption of the receivers was assumed to be equal to A_b of control B animals. Hence, the theoretical area under the plasma concentration-time curve of this amount for unchanged Adriamycin, if the amount had been given intravenously to the receivers, would be:

$$(AUC)_{theoretical} = (A_b)_{control\ B}/(Cl_T)_{control\ B} \quad (9)$$

The fraction of total amount absorbed from the intestine of receivers was estimated according to the following equation:

$$F_{enterohepatic} = (AUC)_{receivers}/(AUC)_{theoretical} \quad (10)$$

Where $(AUC)_{receivers}$ is the area under the plasma concentration-time curve of receivers. The total amount absorbed through enterohepatic recirculation was calculated as $(F_{enterohepatic}) * (A_b)_{control\ B}$.

Statistical analysis

The data are presented as means ± standard deviation. Individual group comparisons were conducted using the two-tailed, paired and unpaired Student's *t*-test (assuming equal or unequal variances) as appropriate. The *F*-tests were used prior to unpaired Student's *t*-tests to examine the equality of two groups variances. All statistical tests were carried out using Microsoft Excel (Version 5.0, Microsoft Corp.).

Results

This study was carried out, using a linked-rat model to determine the role of enterohepatic recirculation in the overall elimination and pharmacokinetics of Adriamycin.

The relevant pharmacokinetic parameters and constants from plasma concentrations of unchanged Adriamycin in control A and B animals and donors are summarized in Table 1. It is apparent that the half-life of the terminal portion of the curve was reduced significantly from 10 h in control A animals to approximately 4 h in control B animals and donors. The total body clearance of control A animals was also significantly lower than the bile duct-cannulated groups. Low levels of Adriamycin associated with the plasma and blood cells of the receivers were detected throughout the experiment with little fluctuation.

By the end of the experiment, approximately 30% (29.89 ± 5.25%) of the dose was recovered in the bile of control B animals. Variable amounts of radioactivity were recovered from the bile of receivers. Based on their biliary elimination during the course of the experiment (12 h), the receivers were divided into two groups, one group with a relatively high elimination (up to 7% of the dose administered to the donors) and the other group with low elimination (up to 0.1% of the dose administered to the donors; Fig. 1). For both groups, the cumulative amount eliminated in the bile was sharply increased between 300 and 400 min following the administration of Adriamycin to donors (Fig. 1).

To investigate whether the variability observed in the amounts eliminated in the bile of the receivers resulted from the variability of the bile flow rates, the flow rates were measured in receivers and control B animals. The results indicated very little fluctuation (15.10 ± 5.7 μl/min in receivers, 12.18 ± 5.3 μl/min in control B animals). Therefore, it was concluded that the lag time was independent of the flow rate. Although the radioactivity recovered from the bile of the receivers was comparatively small, these levels seemed to increase exponentially once the threshold of 5–6 h had been achieved. In fact, the levels attained at the end of the study (12 h) were far from a plateau state suggesting that the biliary elimination of Adriamycin and/or metabolites in the bile of receivers would have continued. Based on the elimination profile of control B animals, it can be assumed that approximately 30% of the administered dose to donors was delivered into the intestine of receivers (approximately 770 μg) from which 64% (492.18 μg) was absorbed (Eq. 10). The cumulative amount excreted in the bile of receivers averaged about 116.9 μg (116.9 ± 75.4 μg), which was approximately 15% of the total

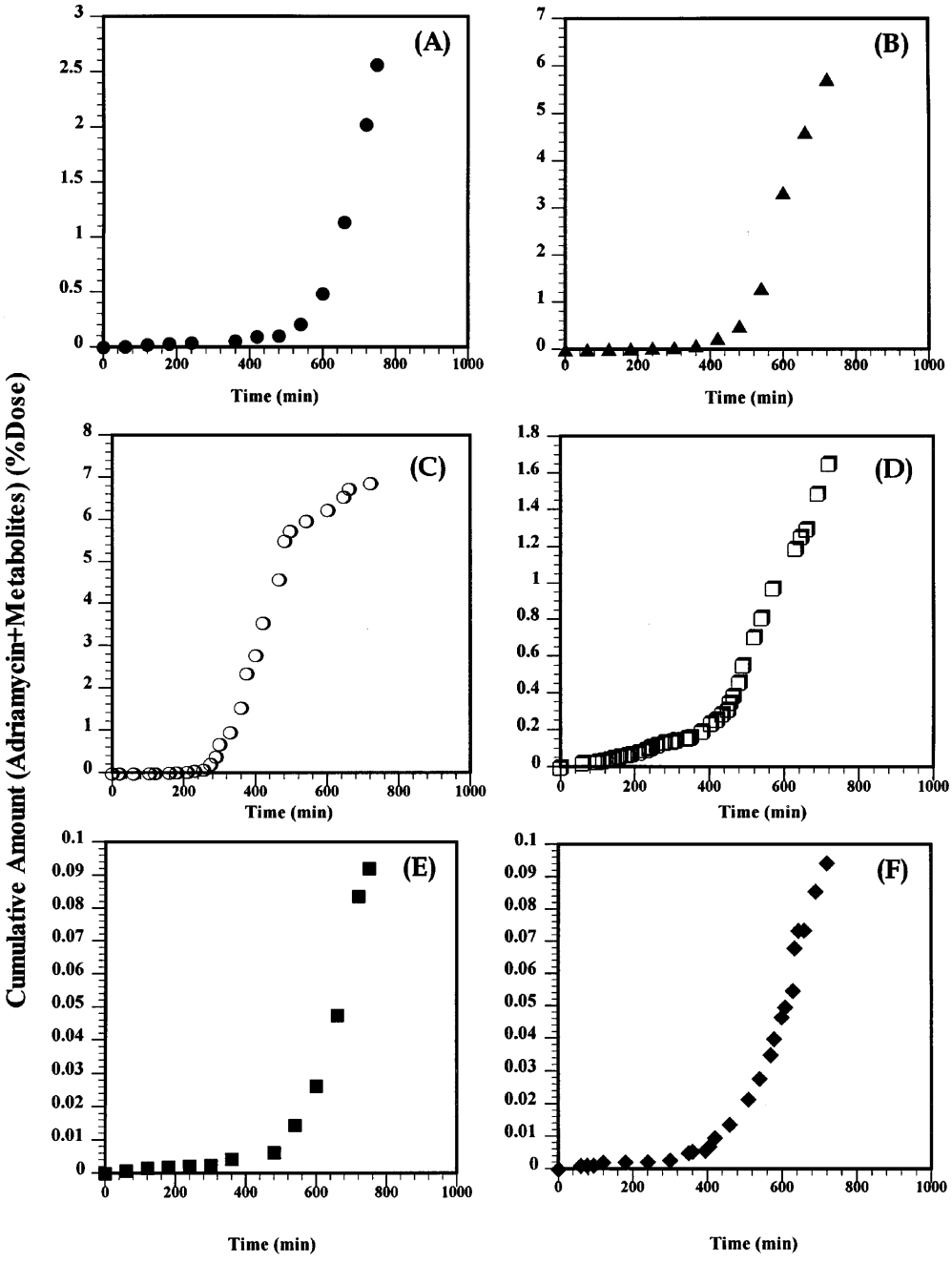

Fig. 1 Profiles of cumulative and total amounts of Adriamycin plus metabolites eliminated in the bile of individual receivers as percentages of the Adriamycin dose administered to the respective donors

amount (770 μg) delivered into their intestine and 24% of the total amount absorbed (Table 2).

Analysis of the bile from control B animals indicated that the primary compound eliminated was unchanged Adriamycin, followed by doxorubicinol and aglycones. The cumulative amounts for biliary elimination of Adriamycin and its major metabolites in receivers are presented in Fig. 2. The fraction of the dose administered to the donors which appeared in the bile of the receivers as unchanged Adriamycin and doxorubicinol was 0.8% and 0.7%, respectively (Table 2). Low levels of doxorubicin aglycone (0.3%) were recovered in the bile of receivers by the end of the experiment. Almost 4.5% of the dose in control B animals was eliminated as doxorubicinol and 2.6% as doxorubicin aglycone (Table 2). Doxorubicinol aglycone was the most abundant metabolite in the bile of the receivers and was nearly equivalent to the levels in control B animals (2.36% in receivers versus 2.9% in control B animals, Table 2) and there was an approximately threefold greater clearance of doxorubicinol aglycone in receivers (5.79 ± 1.91 ml/h) than in control B animals (2.08 ± 0.06 ml/h). However, the biliary clearance of other metabolites and Adriamycin was reduced significantly.

The urinary data revealed that approximately 10% (9.95 ± 0.85%) of the dose administered to control A

Table 2 Biliary elimination of Adriamycin and its major metabolites in control B animals and receivers. Values are means ±SD ($n = 4$ for receivers, and $n = 5$ for control B animals)

Receivers			Control B	
Adriamycin/ metabolites	Cumulative amount excreted (μg)	Fraction of dose excreted[a] $(F_m)_b$	Cumulative amount excreted (μg)	Fraction of dose excreted $(F_m)_b$
Adriamycin	21.25 ± 15.97	0.008 ± 0.003	423.40 ± 25.85	0.149 ± 0.009
Doxorubicinol	18.86 ± 14.03	0.007 ± 0.005	122.31 ± 22.73	0.045 ± 0.008
Doxorubicin Aglycone	9.01 ± 6.38	0.0034 ± 0.001	71.04 ± 14.49	0.0266 ± 0.005
Doxorubicinol Aglycone	58.90 ± 32.73	0.0236 ± 0.01	82.93 ± 25.57	0.029 ± 0.009

[a] The dose here refers to the Adriamycin dose (10 mg/kg, IV) administered to the donors

animals was recovered in the urine within 12 h of the injection of Adriamycin. In donors, however, the recovery was significantly lower (5.95 ± 1.83%, $P < 0.05$). Approximately 6–8% of the dose administered to the control B animals and donors appeared in their urine. By the end of the experiment approximately 0.081% (0.081 ± 0.017%) of the dose given to the donors was recovered in the urine of the receivers. Our results indicate that Adriamycin was the main compound excreted in the urine of donors and control B animals, followed by aglycones and doxorubicinol. In the urine of receivers, however, doxorubicinol aglycone was the main compound. Unidentified peaks constituted approximately 50% of the labeled compounds eliminated in the urine of receivers, and these were assumed to be a combination of deoxy aglycones and the conjugates.

To determine whether the enterohepatic recirculation of Adriamycin and metabolites had resulted in deposition of these compounds in tissues of the receivers, the major organs (heart, liver and kidneys) were collected at the end of the 12 h. Amongst the organs, the liver contained the highest total radioactivity. Our findings indicate that approximately 6.5% of the dose administered

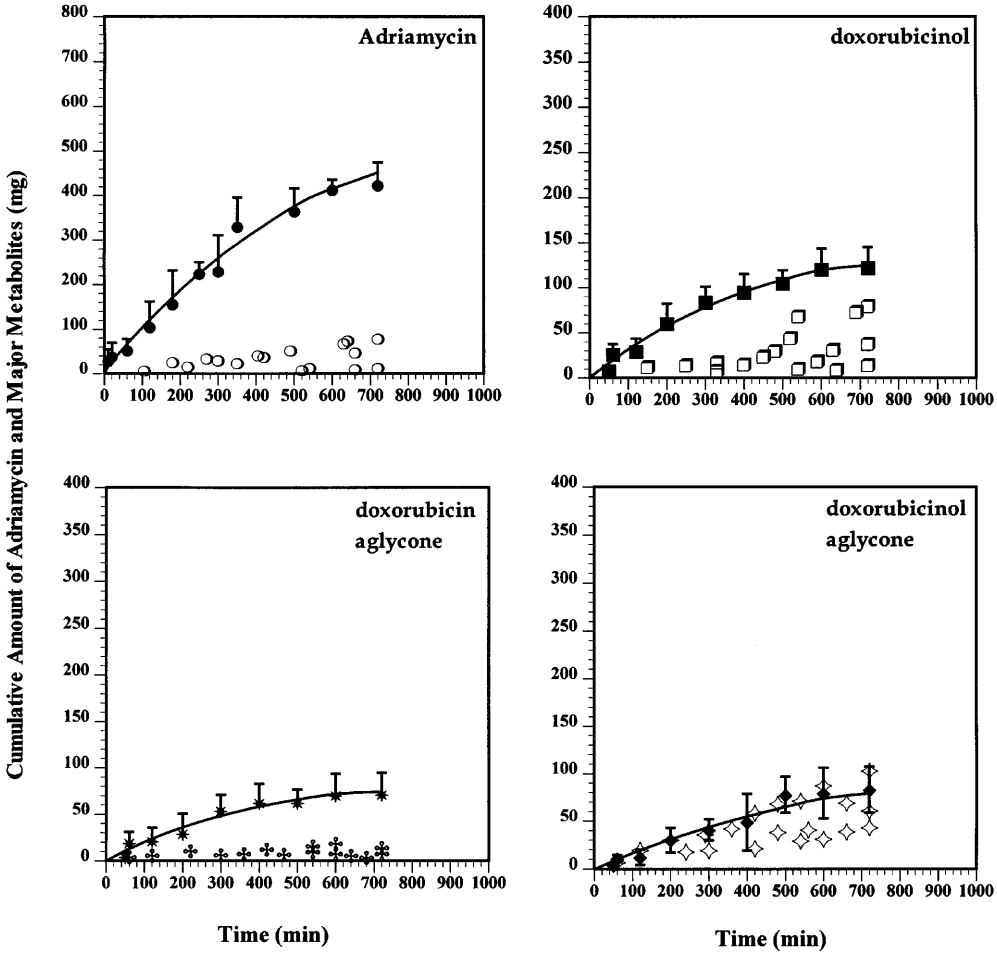

Fig. 2 Cumulative amounts of Adriamycin (●, ○), doxorubicinol (■, □), doxorubicin aglycone (✱, ✣) and doxorubicinol aglycone (◆, ◇) eliminated in the bile of control B animals (*filled symbols*) and receivers (*empty symbols*). The values for control B animals are means ±SD ($n = 5$) and the values for the receivers are from individual animals ($n = 4$)

Table 3 Comparison of the tissue uptake of Adriamycin and metabolites by the major organs (as percentage of dose) following Adriamycin administration to control B animals and donors. The values are amounts (as percentage of dose administered) present in each organ (i.e. in whole liver, heart or a single kidney) and are the means ± SD ($n = 5$ for control B animals and $n = 6$ for donors and receivers)

Group	Kidney	Liver	Heart
Control B	0.63 ± 0.10	5.39 ± 1.10	0.26 ± 0.06
Donor	0.53 ± 0.11	5.77 ± 2.00	0.24 ± 0.07
Receiver [a]	0.01 ± 0.006	0.16 ± 0.06	0.003 ± 0.0004

[a] The dose for the receivers is the amount of Adriamycin (mg) administered to the donors

to donors accumulated in these organs during the 12-h experiment (Table 3). We were able to detect radioactivity in the heart of the receivers, suggesting uptake of the absorbed Adriamycin and/or metabolites by the heart tissue. The receivers' organs contained approximately 0.17% of the dose administered to the donors.

Discussion

We sought to determine whether enterohepatic recirculation of Adriamycin and/or its metabolites occurs using a linked-rat model.

The plasma concentration-time profile of Adriamycin in control B animals and donors demonstrated a half-life of approximately 4 h which was significantly shorter than that of control A animals (10 h). Consequently, the plasma clearance and the overall elimination rate constant of the drug were increased significantly in the bile duct-cannulated animals. These observations suggest a rapid elimination of Adriamycin from the bile duct-cannulated rats which may be attributed to interruption in the enterohepatic recirculation. The plasma concentration-time profile from control A animals, however, failed to show any sudden increase (''secondary hump''), which is the commonly accepted indicator for enterohepatic recirculation. It is important to note that, based on our observation, recirculation occurs gradually, without a sudden elevation of the plasma concentration and after a lag time of approximately 300–400 min.

Within 12 h of injection of Adriamycin (10 mg/kg), approximately 30% (30 ± 2%) of the administered dose appeared in the bile (control B animals). The bile contained mainly unchanged Adriamycin (15% of the dose) followed by doxorubicinol (nearly 5% of the dose) and aglycones (5% of the dose). The remainder (close to 4%) of the dose comprised combined other metabolites, possibly the deoxy forms of the aglycones (deoxy doxorubicin aglycone, deoxy doxorubicinol aglycone) and trace amounts of conjugates (sulfate and glucuronide). To explain the observed threshold of absorption and the interindividual variability, many factors should be considered simultaneously, as no single factor may account for the underlying mechanism(s). The physicochemical nature of the compounds, such as pKa, the aqueous solubility of Adriamycin and doxorubicinol, the hydrophobic nature of the aglycones (owing to the absence of the sugar moiety), the pH of the site of absorption and, therefore, the small intestinal transit time, all may be involved in determining the lag time. It is reasonable to suggest that aglycones, owing to their hydrophobicity, were most likely the compounds that were absorbed from the intestine of the receivers. The lag time of absorption may then be attributed to the formation of the hydrophobic aglycones from Adriamycin in the intestine.

It is well established that aglycones are formed by cleavage of the sugar moiety in highly acidic or basic environments (pH $<$ 4 or pH $>$ 8) [11]. Therefore, it is conceivable that the presence of high levels of Adriamycin or doxorubicinol in the bile and ultimately in the intestine, and the migration through different pH environments of intestine, may lead to the formation of their respective aglycones. Depending upon the rate of migration, which is a function of intestinal motility, Adriamycin and doxorubicinol may reach intestine with the appropriate pH for cleavage of sugar moiety at a later time, which may contribute to the observed threshold. This hypothesis is consistent with the increase in the amount of aglycone in the bile of receivers. The variability and inconsistency of the cumulative amount eliminated in the bile of receivers may be related to the presence of food in the intestine or occasional discontinuity of the bile flow from the donors.

The urine of the receivers contained approximately 0.1% of the dose administered to the donors. The presence of Adriamycin plus metabolites in the urine of the receivers further confirms the presence of these compounds in the systemic circulation of the receivers.

Our findings clearly demonstrate an enterohepatic recirculation for Adriamycin and its metabolites in rats. The presence of low levels of radioactivity detected in the major organs of the receivers is another indication of this recirculation which may contribute to the prolonged exposure to Adriamycin and its metabolites.

References

1. Powis G (1991) Toxicity of free radical forming anticancer agents. Powis G, Hacker M In: The toxicity of anticancer drugs. Pergamon Press, p. 113
2. Leandro J, Dyck J, Poppe D, Shore R, Airhart C, Greenberg M, Gilday D, Smallhorn J, Benson L (1994) Cardiac dysfunction late after cardiotoxic therapy for childhood cancer. Am J Cardiol 74(11): 1152
3. Aviles A, Arevila N, Diaz Maqueo JC, Gomez T, Garcia R, Nambo MJ (1993) Late cardiotoxic toxicity of doxorubicin, epirubicin, and mitoxantrone therapy for Hodgkin's disease in adults. Leuk Lymphoma 11(3–4): 275
4. Praga C, Beretta G, Vigo PL, Lenaz GR, Pollini C, Bonadonna G, Canetta R, Castellani R, Villa E (1979) Adriamycin cardiotoxicity: a survey of 1273 patients. Cancer Treat Rep 63: 827
5. Camaggi CM, Comparsi R, Strocchi E, Testoni F, Angelelli B, Pannuti F (1988) Epirubicin and doxorubicin comparative metabolism and pharmacokinetics. A cross over study. Cancer Chemother Pharmacol 21: 221

6. Speth P, Linssen PCM, Boezman JBM (1987) Cellular and plasma Adriamycin concentrations in long term infusion therapy of leukemic patients. Cancer Chemother Pharmacol 20: 305
7. Robert J (1987) Continuous infusion or intravenous bolus: what is the rationale for doxorubicin administration? Cancer Drug Deliv 4(3): 191
8. Robert J, Vrignaud P, Nguyen-Ngoc T, Llisia A, Mauriac L, Hurteloup P (1985) Comparative pharmacokinetics and metabolism of doxorubicin and epirubicin in patients with metastatic breast cancer. Cancer Treat Rep 69(6): 633
9. Carlo MC, Comparsi R, Strocchi E, Testoni F, Angelelli B, Pannuti F (1988) Epirubicin and doxorubicin comparative metabolism and pharmacokinetics. Cancer Chemother Pharmacol 21: 221
10. Piscitelli SC, Rodvold KA, Rushing DA, Tewksbury DA (1993) Pharmacokinetics and pharmacodynamics of doxorubicin in patients with small cell lung cancer. Clin Pharmacol Ther 53: 555
11. Bouma J, Bejinen JH, Bult A, Underberg WM (1986) Anthracycline antitumor agents: a review of physico-chemical and analytical properties as well as stability. Pharm Wedblad 8: 109
12. Cusack BJ, Young SP, Driskell J, Olson RD (1993) Doxorubicin and doxorubicinol pharmacokinetics and tissue concentrations following bolus injection and continuous infusion of doxorubicin in rabbit. Cancer Chemother Pharmacol 32: 53
13. Arcamone F, Lazzati M, Vicario GP, Zini G (1984) Disposition of ^{14}C-labelled 4'-epidoxorubicin and doxorubicin in the rat. A comparative study. Cancer Chemother Pharmacol 12: 157
14. Tavaloni N, Guarino MA (1980) Biliary and urinary excretion of Adriamycin in anesthetized rats. Pharmacology 20: 256