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Comparative metabolism and elimination of adriamycin and 4'-epiadriamycin in the rat

Trevor W. Sweatman and Mervyn Israel

Departments of Pharmacology and Medicinal Chemistry, The University of Tennessee-Memphis, and The University of Tennessee Cancer Center, Memphis, TN, 38163, USA

Summary. Adriamycin (ADR) and 4'-epiadriamycin (epi-ADR) show comparable biological properties in most in vitro and in vivo test systems. However, in animal studies epi-ADR demonstrates diminished toxicity, especially toward cardiac tissue, compared with ADR. A similar reduction in anthracycline-induced cardiotoxicity with epi-ADR, relative to ADR, is claimed in connection with the clinical evaluation of this agent. The present study was undertaken in an attempt to find a possible pharmacological basis for this observed differential toxicity. Following identical bolus doses (10 mg/kg) of drug administered to rats, no differences were seen in plasma drug levels of the two agents at 4, 7, and 24 h. However, differences in the rate and extent of drug elimination were clearly noted. Thus, following either 24-h infusion or bolus drug administration, parent drugs were eliminated largely in the bile, with the recovery of epi-ADR [50% (infusion) and 40% (bolus) in 54 h and 55 h respectively] exceeding that of ADR [31% (infusion) and 23% (bolus) at the corresponding times]. Recovery of a bolus drug dose in the urine was only 4.8% for epi-ADR vs 3.3% for ADR in 60 h. For each drug the only significant metabolite seen was the corresponding 13-carbinol derivative, adriamycinol (AMNOL) or 4'-epiadriamycinol (epi-AMNOL). The 13-carbinol metabolite represented a larger part of the recovered dose for ADR [15% (infusion) and 18% (bolus) in bile, 0.3% in urine] than for epi-ADR [7% (infusion) and 11% (bolus) in bile, 0.04% in urine]. Epi-ADR was found to be a four fold poorer substrate than ADR for conversion to its 13-carbinol metabolite by aldo-keto reductase enzymes in crude rat liver homogenate preparations. This latter finding provides an explanation for the metabolic and toxicologic differences observed with these agents in whole animals. Previous studies with ADR have reported that degenerative changes in rat cardiac tissue were accompanied by an accumulation of AMNOL during chronic drug administration. Thus, the present study suggests that the reported lower toxicity of epi-ADR compared to ADR for the heart may result from a more extensive elimination of parent drug in bile and urine, as well as diminished metabolic conversion to its 13-carbinol metabolite by ubiquitous aldo-keto reductase enzymes.

Offprint requests to: Mervyn Israel, Department of Pharmacology, University of Tennessee-Memphis, 874 Union Avenue, Memphis, TN 38163, USA

Introduction

In general, the clinical introduction of adriamycin (ADR) in the early 1970s heralded an era of greatly improved therapy of leukemias and various solid tumors. However, the full potential of this agent cannot be realized because of drug-induced cardiac and other toxicities. Consequently, a number of research efforts have been directed toward the identification of ADR analogues with retained or enhanced antitumor activity but diminished toxicity, especially toward the heart.

One such analogue, currently undergoing clinical trial, is 4'-epiadriamycin (epi-ADR), a compound differing from the parent drug only in the stereochemical position of attachment of a hydroxyl group on the 4'-carbon of the aminosugar. A number of comparative studies, both in vitro and in vivo, have demonstrated similarities in biological properties with these epimeric compounds. Thus, epi-ADR and ADR have shown comparable affinity for binding with calf thymus DNA and in the inhibition of DNA and RNA polymerases and [^{3}H]-thymidine incorporation in mouse embryo fibroblasts [2, 15]. Epi-ADR has proven more effective than ADR at RNA synthesis inhibition in L1210 cells [27] but comparable to ADR in cytotoxic activity against a range of murine and human cell lines [19]. In vivo studies have demonstrated that the epimers show comparable antitumor activity in mice for ascitic and solid sarcoma 180, Gross leukemia, L1210 and P388 leukemias, and mammary carcinoma [4], although epi-ADR was found to be more active against Lewis lung carcinoma and against the MS-2- and MSV-induced sarcomas [12]. A recent review of clinical trials with epi-ADR found this analogue to possess a spectrum of antitumor activity similar to that of ADR [13].

Studies of acute and chronic toxicity have, however, highlighted important differences between the two drugs. Acute studies in mice, both normal and tumor-bearing, revealed a lesser toxicity for epi-ADR than ADR [12]. Most notable is the reported reduction in morphological changes in cardiac tissue seen in both rabbits [12], and rats and dogs [9], indicative of a reduced toxicity of epi-ADR towards this organ. The present study was undertaken in an attempt to determine whether there may be a pharmacologic rationale for the reported differences in toxicity between ADR and epi-ADR.

Materials and methods

Animals. Female Sprague-Dawley rats (200–300 g) were

used throughout this study. For the purpose of surgery, cardiac puncture, or i.v. dosing via the tail vein, animals were temporarily anesthetized by inhalation of methoxyflurane (Metofane, Pitman-Moore, Washington Crossing, NJ, USA).

For biliary studies, the external jugular vein and bile duct were both exposed and cannulated with polyethylene tubing (PE10, Clay Adams, Parsippany, NJ, USA). The cannulae were passed posteriorly under the skin to emerge from the tail approximately 0.5 inch from the base. Cannulae were secured with ties and wounds were closed with surgical clips. A coiled steel spring was fitted over the animal's tail, attached to the base with surgical steel, and secured to the cage wall; this protected the cannulae while allowing the rat relative freedom of movement and access to food and water throughout the experiment. Patency of the i.v. cannulae was maintained by saline infusion (10 ml/24 h). Animals were allowed to recover from surgery overnight prior to drug administration. Drug (ADR or epi-ADR, 10 mg/kg), dissolved in saline was administered either as an i.v. push over 3 min or by 24-h infusion using a syringe-drive pump (Harvard Apparatus Co., Harvard, Mass, USA). All i.v. lines were cleared with saline, and bile samples, protected from light, were collected at various times after dosing (1, 2, 3, 5, 7, 9, 24, 33, 48, and 54–55 h) and were stored at −70 °C pending analysis.

For urinary studies, animals acclimated overnight in metabolism cages (Fisher Scientific, Pittsburgh, Pa, USA) were given an i.v. bolus dose of ADR or epi-ADR, 10 mg/kg, via the tail vein and were replaced in the cages for the separate collection of urine and feces at preselected time intervals (6, 24, 36, 48, and 60 h). Samples were again stored frozen (−70 °C) pending analysis.

Plasma studies were conducted on animals given an i.v. bolus dose of drug (ADR or epi-ADR, 10 mg/kg) via the tail vein. Blood samples (0.5–3 ml) were obtained by cardiac puncture at various times after dosing (1, 2, 4, 7, and 24 h), and plasma, resulting from centrifugation, was stored frozen (−70 °C) prior to extraction and analysis.

In vitro studies. In vitro studies were conducted on 100000-*g* supernatant fractions of rat liver homogenates prepared in 3 volumes of 2.5 m*M* potassium phosphate buffer, pH 7.5, containing 0.25 *M* sucrose and 5 m*M* 2-mercaptoethanol. The protein content of the supernatants was determined by the method of Lowry et al. [22]. Drugs (50 μmol ADR or epi-ADR) were incubated with supernatant (containing 1 or 2 mg protein) in the presence of a saturating concentration of NADPH (1 mM, Sigma, St. Louis, Mo, USA) for 30 or 60 min at pH 8.5 (0.25 *M* Tris HCl). Enzyme activity was stopped by the addition of 0.3 ml isopropanol and placement of the samples on ice. The resulting solutions were saturated with sodium chloride and centrifuged to separate the organic layer, which was collected and evaporated to dryness under dry nitrogen at 37 °C. Samples were stored at −20 °C pending analysis. Daunorubicin (DNR), known to be an excellent substrate for the hepatic aldo-keto reductase enzymes, was used as a positive control and was incubated for 1 or 5 min at a concentration of 50 or 100 μmol under identical conditions.

Sample processing. Bile, urine, and plasma samples (0.1–1 ml) were adjusted to pH 8.5 with an equal volume of 0.05 *M* Tris buffer. DNR (250 ng/sample) was added as internal standard and the samples were extracted with 2×4 volumes of either chloroform:1-propanol (3/1 v/v, bile, urine) or ethyl acetate:1-propanol (9/1 v/v, plasma). The combined organic phases for each sample were evaporated to dryness under dry nitrogen at 37 °C. Fecal samples were allowed to soak in buffer prior to extraction as for urine.

A portion of each of the bile samples collected from 6 through 24 h from animals treated with epi-ADR was incubated with an equal volume of 0.05 *M* sodium acetate buffer, pH 5.0, for 1 h at 37 °C in the presence or absence of β-glucuronidase (Sigma, St. Louis, Mo, USA, >20 U/sample). Samples were prepared for analysis by extraction, as noted above. Enzyme viability was established using the colorimetric change in the liberation of phenolphthalein from its glucuronide conjugate according to Sigma Technical Bulletin 325.

Analytical conditions. Samples from in vivo studies were analyzed in methanolic solution (100–150 μl) by reversed-phase HPLC using a microprocessor-controlled automated dual pump system fitted with a 10 cm×8 mm internal diameter (ID) phenyl-Radialpak column mounted in a Z-Module radial compression assembly (all equipment Waters Associates, Milford, Mass, USA). The mobile phase consited of 0.05 *M* ammonium formate buffer, pH 4.0, and spectral grade acetonitrile (American Burdick and Jackson, Muskegon, Mich, USA) at a flow rate of 3.0 ml/min. Gradient conditions were as follows: initial conditions 75% buffer:25% acetonitrile; at 10 min, 35% buffer:65% acetonitrile (linear gradient), with these conditions held for 5 min before resetting for the next sample injection. The column eluate was monitored by flow fluorometry (Schoeffel Model FS-970, Kratos Schoeffel Instruments, Ramsey, NJ, USA), set for excitation at 482 nm and equipped with a 550-nm emission cut-off filter. Retention times (min) of parent drugs and 13-carbinol metabolites were as follows: ADR 8.47; epi-ADR 9.46; AMNOL 5.25; epi-AMNOL 6.26. Peak quantitation was conducted by reference to standard curves of blank samples to which authentic standards had been added, with correction for the recovery of internal standard. The limit of accurate quantitation for this assay methodology was 5 ng/injection, with smaller peaks being recorded but not integrated.

Samples from in vitro studies, including those containing DNR, were similarly analyzed by means of reversed-phase HPLC. However, incubation of either ADR or epi-ADR in pH 8.5 buffer at 37 °C for an extended period, as in these incubation studies, results in a small (<5%) but significant drug degradation, with the formation of a polar product which is difficult to separate from the respective 13-carbinol metabolites using this analytical technique. For this reason, extracts of liver supernatants incubated with ADR or epi-ADR were additionally subjected to normal-phase HPLC [20] to allow adequate resolution of parent drug, metabolite, and breakdown product. Separation was achieved on a Partisil PXS 10/25 PAC column (25 cm×4.6 mm ID, Whatman Inc., Clifton, NJ, USA), using a linear gradient with a mobile phase of chloroform vs a mixed solvent consisting of chloroform:methanol:acetic acid:water (85:15:5:1.5 by volume), at a flow rate of 3.0 ml/min. Initial conditions were 70% chloroform:30% mixed solvent; final conditions of 100% mixed solvent

were achieved over 20 min. Samples were detected by flow fluorescence detection, as noted above, and the extent of metabolism was determined by comparison of peak areas of authentic standards of parent drug and product. Under these conditions the retention times (min) of parent compounds and repective metabolites were as follows: ADR 12.08; AMNOL 16.08; epi-ADR 11.31; epi-AMNOL 15.30.

Results

The cumulative biliary elimination of anthracycline fluorescence following i.v. administration of ADR or epi-ADR, 10 mg/kg, to Sprague-Dawley rats is shown in Fig. 1. The rate of elimination of drug in the bile was calculated by plotting the logarithm of percentage of recovered dose remaining for excretion vs time. Data for mean excretion of parent drug and metabolite following bolus administration are shown in Table 1, for recovery of drug and metabolite in bile following 24-h influsion in Table 2.

Analysis of bile samples from rats given ADR or epi-ADR, 10 mg/kg, revealed the existence of two distinct fluorescent peaks in both cases. In each instance the greater part of the fluorescence was attributable to unchanged parent drug; the lesser fluorescent signal eluted at a retention time idential to the corresponding authentic 13-carbinol metabolite. No other significant metabolic products were detected, and no change was seen in the chromatograms of epi-ADR samples incubated with β-glucuronidase.

Following a bolus injection of ADR, biliary elimination of drug was slow, with 9% of the dose recovered by 2 h and 28% by 55 h, the last time-point included in this study. At this time, low levels of anthracyclines were still detectable in the bile. Unchanged parent drug accounted for the major part (82%) of recovered fluorescence, the remainder being attributable to AMNOL. Although the overall recovery of total drug (36% in 54 h) was increased when ADR was administered by infusion, as opposed to bolus injection, the metabolic pattern was similar with 15% of recovery as AMNOL and the remainder as unchanged drug.

Biliary elimination of epi-ADR was similar to that of ADR during the initial 3 h following administration; thereafter, recovery of anthracycline fluorescence exceeded that of ADR, with 45% of the dose detected in bile by 55 h. Trace levels of aglycones were detected in some samples, but unchanged epi-ADR (89% of the total) and epi-AMNOL (almost 11% of the total) predominated. Thus, recovery of epi-AMNOL was similar to that of AMNOL, although its initial excretion was somewhat slower (0–3 h). Like ADR, the metabolism of epi-ADR was unchanged when given by 24-h infusion (Table 2); under these conditions, 54% of the administered dose was recovered in the bile by 54 h, consisting of unchanged drug (93% of the total) and epi-AMNOL (7% of the total).

Urinary elimination proved to be a minor metabolic pathway for both drugs, with only 3.5% of the administered dose of ADR and 4.8% of the dose of epi-ADR being recovered by this route in 60 h (Table 3). As anticipated, elimination took place predominantly in the first 24 h fol-

Table 1. Comparison of biliary rate constants for elimination of parent drug and 13-carbinol metabolite following i.v. bolus administration of ADR or epi-ADR (10 mg/kg) to Sprague-Dawley rats

Compound	K_{el} (/h)[a]	Time (h)
ADR	0.04822±0.0125	2–33
epi-ADR	0.08629±0.0512	2– 9
	0.04046±0.0143	9–33
AMNOL	0.04510±0.0189	2–33
epi-AMNOL	0.04400±0.0050	2–33

Results expressed as mean ± SD (n = 6 in each group)
[a] Biliary rate constant for elimination

Table 2. Recovery of parent drugs and 13-carbinol metabolites in bile following 24-h infusion of ADR or epi-ADR (10 mg/kg) to Sprague-Dawley rats

Time (h)	ADR (AMNOL)	epi-ADR (epi-AMNOL)
0–24	21.9±7.8	36.2±16.3
	(3.2±1.5)	(2.4±0.6)
0–48	30.2±9.4	49.0±17.5
	(5.1±1.9)	(3.9±1.0)

Results expressed as percentage of dose (mean ± SD, n =6)

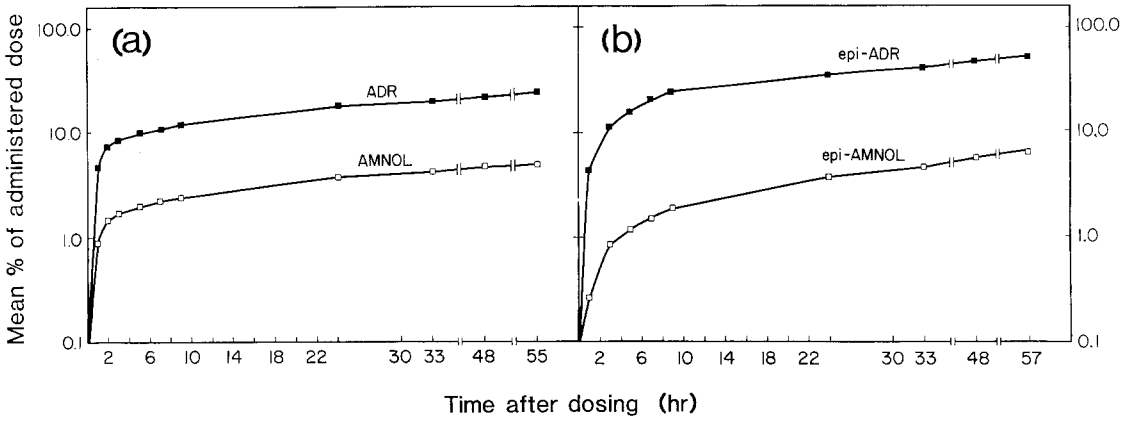

Fig. 1a, b. Mean cumulative biliary elimination of parent drug and metabolite in Sprague-Dawley rats following an i. v. bolus dose of 10 mg/kg of **a** ADR or **b** epi-ADR. n=6 for each group

Table 3. Cumulative elimination of anthracycline in rat urine following an i.v. bolus dose (10 mg/kg) of ADR or epi-ADR

Time (h)	ADR	epi-ADR	AMNOL	epi-AMNOL
0– 6	2.08 ± 1.26	2.43 ± 0.29	0.22 ± 0.13	0.024 ± 0.047
0–24	2.77 ± 1.27	3.90 ± 0.25	0.26 ± 0.13	0.030 ± 0.045
0–60	3.33 ± 1.46	4.83 ± 0.34	0.30 ± 0.15	0.044 ± 0.043

Figures represent percentage of dose (mean ± SD, $n = 6$ in each group)

Table 4. Concentration of parent drug in the plasma of rats following an i.v. bolus dose (10 mg/kg) of ADR or epi-ADR

Time (h)	Concentration (ng/ml)	
	ADR	epi-ADR
1	345.6 ± 230.6	413.3 ± 152.4
2	188.2 ± 71.5[a]	269.1 ± 66.4[a]
4	169.0 ± 64.8[a]	183.4 ± 54.9[a]
7	107.9 ± 41.2	124.4 ± 51.1
24	37.0 ± 14.6	39.1 ± 12.6

Results expressed as mean ± SD ($n = 5$ in each group)

[a] Low levels of the corresponding 13-carbinol and aglycone metabolites were detected in some samples

lowing drug administration, with only low urinary levels detected beyond 48 h. A significant difference ($P < 0.01$) in the extent of metabolism was detectable: with epi-ADR, urinary fluorescence was almost exclusively unchanged drug (99%, with 1% as epi-AMNOL), while with ADR, 10% of the urinary fluorescence was due to AMNOL.

Fecal analysis revealed largely anthracycline aglycone metabolites, although trace levels of both parent drugs were detectable (with 6% of the administered dose accounted for in 60 h). The inconsistency between these findings and the biliary recovery is probably a result of metabolic breakdown to nonfluorescent compounds by intestinal microflora.

Parent drugs were the predominant anthracyclines detected in all plasma samples, their levels declining in a biphasic manner during the course of the study. The sampling times (1, 2, 4, 7, and 24 h), too widely spaced for the determination of kinetic data, were chosen to allow a comparison of circulating levels of the two anthracyclines during the tissue equilibration and elimination phases. Although the standard deviations of the parent drug plasma levels are high (typically ±30%), the mean values for samples obtained at 1 and 2 h after drug administration suggest higher plasma drug levels in animals given epi-ADR than in those given ADR. At the later sampling times, the plasma drug concentrations do not show this difference (Table 4). The two drugs showed comparable circulating metabolite levels, with occasional low (< 50 ng/ml) levels of respective 13-carbinol metabolites, and trace amounts of the aglycone 7-deoxy-13-dihydroadriamycinone. These minor metabolites reached their highest levels at 2 or 4 h and declined thereafter to levels at or below the limit of detectability (5 ng/ml).

The enzyme activity of liver supernatants against ADR, epi-ADR, and DNR as substrate is shown in Table 5. DNR, as noted earlier, was included in this study for comparison since it is known to be a superior substrate for the aldo-keto reductase enzymes; in this respect it acted as a positive control and, as expected, showed more extensive metabolism than either ADR or epi-ADR. All three anthracyclines were metabolized in a time- and enzyme-dependent manner, with the extent of metabolism after exposure to twice the enzyme level or twice the incubation period showing the appropriate increase. No drug metabolism took place in the absence of enzyme or NADPH cofactor. The data clearly show that ADR was a better substrate for the crude aldo-keto reductase preparations than was epi-ADR, the rate of metabolism being some 4 times greater for ADR than its epimer.

Table 5. Relative substrate specificity at pH 8.5 of DNR, ADR, and epi-ADR toward crude rat liver aldo-keto reductase preparation

Drug	nmol/min/mg protein
DNR	6.55 ± 0.97
ADR	0.133 ± 0.029
epi-ADR	0.029 ± 0.007

Results represent mean ± SD for liver homogenates from three animals. Samples were analyzed in duplicate by HPLC. Values for each animal were calculated from three incubations, including 2 × incubation time and 2 × enzyme concentration. Protein determinations were carried out according to the method of Lowry et al. [22]

Discussion

Results presented here confirm previous reports [3, 10] that epimerization of the glycosidic C-4' alcohol function does not substantially alter the route of elimination of the anthracyclines in rats. Both ADR and epi-ADR were recovered extensively in the bile, with only limited urinary excretion being seen. However, the present study has uncovered differences in the overall recovery and extent of metabolism of the two epimers. The total recovery of epi-ADR in the bile clearly exceeded that of ADR following administration of equal doses of drugs either by bolus or infusion. Biliary levels of epi-AMNOL in epi-ADR-treated rats were consistently lower than those of the corresponding 13-carbinol metabolite in ADR-treated animals. These compounds were the only significant metabolites detected in the present study.

The mean rate of biliary elimination of ADR over the period 2–33 h (0.04822 K_{el}/h; Table 1) was similar to that reported previously [21]. While elimination of ADR was essentially biphasic, with a rapid initial rate through the first 60 min and a more gradual biliary recovery thereafter, excretion of epi-ADR showed three phases. In common with ADR, biliary excretion of epi-ADR was initially rapid (0–1 h). An intermediate elimination rate was evident from 2–9 h, followed, from 9–33 h, by drug excretion at a rate comparable with that seen for ADR (Table 1). Recovery of the two agents over the period 0–3 h was similar (8.5% of the ADR dose vs 11.1% of the epi-ADR dose). Consequently, the overall greater recovery of epi-ADR than ADR in the bile must be accounted for by a greater rate and extent of elimination over the intermediate period of 2–9 h. As shown in Table 2, differences in the recovery

of ADR and epi-ADR in bile were also apparent when the drugs were given by 24-h infusion rather than by bolus administration (Fig. 1). Thus, the data indicate that there may be differences in tissue distribution and/or hepatic extraction between the two agents. Although standard deviations were large, plasma levels of drug observed at 1 and 2 h following administration were indicative of higher circulating drug levels in animals given epi-ADR than in comparably treated rats receiving ADR (Table 4). As a result, we favor reduced tissue distribution as the explanation for the observed greater biliary elimination of epi-ADR than ADR.

The results presented here on the differences between ADR and epi-ADR with respect to plasma drug levels and extent of biliary excretion in rats are at variance with earlier reports. Thus, Arcamone et al. [3] reported lower plasma levels of epi-ADR than ADR at 40 min and 4 h following administration of the drugs; however, the dose used in this study was only 1 mg/kg. On the other hand, Broggini and colleagues [10] reported comparable plasma levels and biliary recovery, albeit over a shorter time period than used here, in tumor-bearing mice given the drugs at doses 50% greater than in the present study.

Appearance of drug in the urine following an i.v. bolus dose was limited but comparable (3.5% for ADR vs 4.8% for epi-ADR), with a significant ($P < 0.01$) difference in the recovery of the 13-dihydro metabolites by this route. In common with biliary metabolism, the levels of epi-AMNOL in urine were lower than those of AMNOL. Overall recovery by this route was less than that reported previously by Arcamone et al. [3], an observation that probably reflects the greater recovery of drug and metabolites in the bile in the present study.

Data produced by the in vitro studies with rat liver supernatants support the observed differences in the extent of drug metabolism to the respective 13-carbinol derivatives. Previous studies have purified and characterized the aldo-keto reductase enzyme responsible for this conversion [1, 17]. These enzymes, located within the cytosolic fraction, are found in a number of tissues, but occur mainly in the liver and kidney. Ahmed et al. [1] have shown that in rabbit and man, there are at least two distinct pH optima (6.0 and 8.5) for the metabolism of anthracyclines by these enzymes; in the mouse and rat, however, activity is not detectable at the lower pH. The present study confirms earlier observations, both in vitro [1] and in vivo [6], that reduction of the methyl ketone group at C-13 of DNR occurs in preference to that of the hydroxymethyl ketone group at the same position in adriamycin. In this respect, the substrate specificity found in the present study with crude liver supernatants (ADR 0.133, DNR 6.55 nmol/min/mg protein) is similar to that observed by Ahmed and colleagues [1] using a purified enzyme preparation (ADR 0.19, DNR 5.8 nmol/min/mg protein). In the present study, epi-ADR proved to be a poorer substrate for this enzymatic conversion than ADR, since production of epi-AMNOL was 4 to 5 times less than that of AMNOL. While this observation may explain the lower levels of epi-AMNOL noted in bile and urine, it might be of greater significance in understanding the reduced cardiotoxicity noted for epi-ADR, compared with ADR, during chronic toxicity studies.

Del Tacca and coworkers [14], in a study involving the administration of ADR (2 mg/kg i.v.) to rats once a week for 3 weeks, noted that while plasma levels of ADR and AMNOL (and ADR levels in caridac tissue) were unchanged by this treatment schedule, levels of AMNOL in heart tissue increased significantly during the study. This increase was accompanied by changes in ECG parameters and there was histological evidence of tissue degeneration at the termination of the study. The presence of AMNOL within the cardiac tissue was thought to be due to intracellular production from ADR by reductase enzymes which have been reported in this organ [26], rather than to uptake from the circulation, since the polarity of AMNOL significantly reduces the cellular uptake of this metabolite in vitro compared with the parent compound [7]. Both ADR and AMNOL may be metabolized, within the heart and other tissues, to respective aglycone metabolites via a process involving anthracycline semiquinone intermediates and the production of cytotoxic superoxide radicals [5, 8, 24]. These short-lived radicals have been implicated in the damage and subsequent degeneration of cardiac tissue [16, 24]. While tissue levels of epi-ADR have been reported in acute studies [3, 12], these experiments did not quantify metabolites but relied upon radioactivity or total fluorescence as a measure of drug concentration. Although chronic studies in several animal species have shown that epi-ADR produces somewhat less cardiotoxicity than ADR [12, 13], no comparative tissue levels have been reported during such long-term exposure. The finding that epi-ADR is a 4 to 5 times poorer substrate than ADR for conversion into its 13-carbinol metabolite might predict lower levels of epi-AMNOL within cardiac tissue. The diminished enzymatic reduction of epi-ADR compared with ADR, coupled with the increased elimination of this epimer in rat bile, as described in this report, could serve to explain the reduced experimental cardiotoxicity noted with this compound.

Consistent with this line of thought, clinical studies with epi-ADR have noted the existence of glucuronide conjugates of both epi-ADR and epi-AMNOL [11]. Such metabolites have not been detected following ADR administration. Overall clearance of epi-ADR in man is greater than that of ADR [11, 23]. Toxicity studies have reported that in man as in animal models, epi-ADR induces less acute toxicity (vomiting, alopecia, myelosuppression) than ADR and also less cardiotoxicity, as evidenced by radionuclide scans and myocardial biopsies [18]. We have found that the aldo-keto reductase activity of human liver shows the same relative substrate affinity for ADR and epi-ADR as was evident in the present rat studies [25]. Thus, the possibility exists that the diminished clinical cardiotoxicity of epi-ADR, like that observed in the rat, may be due to the combination of greater drug clearance and reduced metabolism to epi-AMNOL.

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References

1. Ahmed NK, Felsted RL, Bachur NR (1978) Heterogeneity of anthracycline antibiotic carbonyl reductases in mammalian liver. Biochem Pharmacol 27: 2713
2. Arcamone F, DiMarco A, Casazza AM (1978) Chemistry and pharmacology of new antitumor anthracyclines. In: Umezawa H et al. (eds) *Advances in Cancer Chemotherapy*. Jpn Sci Soc Press, Tokyo/Univ Park Press, Baltimore, p 297

3. 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
4. Arcamone F, Penco S, Vigevani A, Redaelli S, Franchi G (1975) Synthesis and antitumor properties of new glycosides of daunomycinone and adriamycinone. J Med Chem 18: 703
5. Bachur NR, Gordon SL, Gee MV, Kon H (1979) NADPH cytochrome P450 reductase activation of quinone antitumor agents to free radicals. Proc Natl Acad Sci USA 76: 954
6. Bachur NR, Hildebrand RC, Jaenke RS (1974) Adriamycin and daunorubicin disposition in the rabbit. J Pharm Exp Ther 191: 331
7. Bachur NR, Steele M, Meriwether WP, Hildebrand RC (1976) Cellular pharmacodynamics of several anthracycline antibiotics. J Med Chem 19: 651
8. Berlin V, Haseltine WA (1981) Reduction of adriamycin to a semiquinone free radical by NADPH cytochrome P450 reductase produces DNA clevage in a reaction mediated by molecular oxygen. J Biol Chem 256: 4747
9. Bertazzoli C, Rovero C, Ballerini L, Lux B, Balconi F, Antongiovanni V, Magrini U (1985) Experimental systemic toxicology of 4'-epidoxorubicin, a new less cardiotoxic anthracycline antitumor agent. Toxicol Appl Pharmacol 79: 412
10. Broggini M, Colombo T, Martini A, Donelli MG (1980) Studies on the comparative distribution and biliary excretion of doxorubicin and 4'-epidoxorubicin in mice and rats. Cancer Treat Rep 64: 897
11. Camaggi CM, Strocchi E, Martoni A, Angelelli B, Compassi R, Pannuti F (1985) Epirubicin plasma and blood pharmacokinetics after single iv bolus in advanced cancer patients. Drug Exp Clin Res 11: 285
12. Casazza AM, DiMarco A, Bertazzoli C, Formelli F, Giuliani F, Pratesi G (1978) Antitumor activity, toxicity and pharmacological properties of 4'-epiadriamycin. In: Siegenthaler W, Luthy R (eds) Current Chemotherapy Vol 2, Am Soc Microbiol, Washington, DC, p 1257
13. Cersosimo RJ, Hong WK (1986) Epirubicin: a review of the pharmacology, clinical activity and adverse effects of an adriamycin analogue. J Clin Oncol 4: 425
14. Del Tacca M, Danesi R, Ducci M, Bernardini C, Romanini A (1985) Might adriamycinol contribute to adriamycin-induced cardiotoxicity? Pharm Res Commun 17: 1073
15. DiMarco A, Casazza AM, Dasdia T (1977) Changes of activity of daunorubicin, adriamycin and stereoisomers following the introduction or removal of hydroxyl group in the amino sugar moiety. Chem Biol Interact 19: 291
16. Doroshow JH (1983) Effect of anthracycline antibiotic on oxygen radical formation in rat heart. Cancer Res 43: 460
17. Felsted RL, Richter DR, Bachur NR (1977) Rat liver aldehyde reductase. Biochem Pharmacol 26: 1117
18. Ganzina F, DiPietro N, Magni O (1985) Clinical toxicity of 4'-epidoxorubicin (epirubicin). Tumori 71: 233
19. Hill BT, Whelan RDH (1982) A comparison of the lethal and kinetic effects of doxorubicin and 4'-epidoxorubicin in vitro. Tumori 68: 29
20. Israel M, Pegg WJ, Wilkinson PM, Garnick MB (1978) Liquid chromatographic analyses of adriamycin and metabolites in biological fluids. J Liq Chromatogr 1: 795
21. Israel M, Wilkinson PM, Pegg WJ, Frei III E (1978) Hepatobiliary metabolism and excretion of adriamycin and N-trifluoroacetyladriamycin-14-valerate in the rat. Cancer Res 38: 370
22. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265
23. Martoni A, DiMarco AR, Camaggi CM, Strocchi E, Fruet F, Marraro D, Giovannini M, Tomasi L, Pacciarini MA, Pannuti F (1983) Doxorubicin and 4'-epidoxorubicin: clinical experience and pharmacokinetics. Drug Exp Clin Res 9: 767
24. Nohl H, Jordan W (1983) OH· generation by adriamycin semiquinone and H_2O_2, an explanation for the cardiotoxicity of anthracycline antibiotics. Biochem Biophys Res Commun 114: 197
25. Sweatman TW, Ahmed NK, Israel M (1986) Relative activity of daunorubicin, adriamycin and 4'-epiadriamycin with human liver reductases. Proc Am Assoc Cancer Res 27: 247
26. Von Wartburg JP, Wermuth B (1980) Aldehyde reductase. In: Jakoby WB (ed) Enzymatic basis of detoxication Vol 1, Academic, New York, p 249
27. Wilson RG, Kalonaros V, King M, Lockwood R, McNeill M (1981) Comparative inhibition of nuclear RNA synthesis in cultured mouse leukemia L1210 cells by adriamycin and 4'-epiadriamycin. Chem Biol Interact 37: 363

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