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ORIGINAL ARTICLE

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Effect of dexrazoxane on doxorubicin pharmacokinetics in young and old rats

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Abstract *Purpose*: Although dexrazoxane (ICRF-187) is used clinically to protect against doxorubicin cardiotoxicity, the age-related effect of dexrazoxane on doxorubicin pharmacokinetics has not been well studied. *Methods*: We therefore examined the effect of pretreatment with dexrazoxane (50 mg kg^{-1} i.p. 1 h prior to administration of doxorubicin 2 mg kg^{-1} i.v. bolus) on doxorubicin and doxorubicinol pharmacokinetics in Fischer 344 rats at 5 months of age (young adult) and 22 months of age (old). *Results*: Dexrazoxane had no major effects on doxorubicin or doxorubicinol pharmacokinetics in plasma or heart in either young or old rats. However, age had significant effects on anthracycline pharmacokinetics. Early plasma concentrations were increased and 2systemic clearance of doxorubicin was decreased in old compared with young rats. Cardiac concentrations of doxorubicin (AUC) were significantly increased in old rats. In addition cardiac doxorubicinol concentrations (AUC 0–72 h) were increased by over 80% in old compared to young rats. *Conclusion*: The results suggest that dexrazoxane does not alter doxorubicin pharmacokinetics. In contrast, aging in the rat model is associated with altered doxorubicin and doxorubicinol pharmacokinetics, in particular in the heart. These changes could increase the risk of anthracycline cardiotoxicity with age.

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Introduction

Cardiotoxicity is a cumulative, dose-limiting adverse effect of anthracyclines such as doxorubicin and daunorubicin [1, 2]. Hence, some patients may not be able to receive an adequate cumulative dose of anthracycline without the risk of cardiac injury. Factors that increase the risk of anthracycline-induced cardiomyopathy include cumulative dose, dosage schedule [3], mediastinal radiotherapy [4], prior cardiac disease [5] and patient age [3]. It is not known why old age is an independent risk factor for cardiotoxicity, but an age-dependent decrease in doxorubicin clearance in the early phase after drug administration may contribute [6, 7]. This raises the possibility that age-related changes in doxorubicin disposition may increase the risk of cardiotoxicity. Dexrazoxane (ICRF-187), the only approved agent for prevention of anthracycline cardiotoxicity, lowers the risk of cardiotoxicity in patients with breast cancer [8] and prevents cardiotoxicity in animals including the rat [9, 10]. There is, however, a paucity of data available concerning the effect of dexrazoxane on the pharmacokinetics of doxorubicin, particularly in relation to aging. Studies in the rat have demonstrated increased uptake of doxorubicin in the heart following dexrazoxane pretreatment [11]. In patients with breast cancer, dexrazoxane does not alter the pharmacokinetics of doxorubicin [12]. Although drug-drug interactions due to alteration of metabolism also occur in old age [13], it remains unknown whether the effect of dexrazoxane on anthracycline disposition is age-dependent. Accordingly, this study was designed to test the hypothesis that the effect of dexrazoxane on doxorubicin pharmacokinetics is altered in old age and, secondly, that the disposition of doxorubicin and doxorubicinol is age-dependent in the Fischer 344 rat.

Materials and methods

Protocol

Male Fischer 344 rats aged 5 months and 22 months were purchased from Harlan Sprague-Dawley (Indianapolis, Ind.) and acclimatized under observation for approximately 1 week prior to inclusion in the study. The welfare of the animals was protected and the local animal care and use committee approved the study protocol. There were two treatment groups within each age cohort. One treatment group received doxorubicin (Adriamycin RDF, Pharmacia & Upjohn, Kalamazoo, Mich.) 2 mg kg^{-1} as a single-dose bolus injection into a left hind leg vein over 1 min. The second group was pretreated with 50 mg kg^{-1} dexrazoxane (Zinecard, Pharmacia & Upjohn) given by i.p. injection 30 min prior to the i.v. bolus injection of doxorubicin as described above. Animals were killed 1, 4, 8, 24, 30, 48, and 72 h after the doxorubicin injection by administration of 20 mg sodium pentobarbital (Lenexa, Kansas) into a right thigh vein. Immediately after the animals had been killed, the chest cavity was opened and blood samples were aspirated from the left ventricular cavity using a 23G needle. Blood was then removed from the systemic circulation by perfusing 0.9% NaCl into the inferior vena cava. Transmural tissue samples (100–150 mg) were then resected from the left ventricle. Plasma and tissue samples were stored at –70°C until assay for doxorubicin and doxorubicinol.

Preparation of standards

Doxorubicinol was prepared from rabbit kidney cytosol by a method similar to that previously described for preparation of daunorubicinol [14]. Doxorubicin, daunorubicin (Sigma, St. Louis, Mo.) and doxorubicinol were made as chromatographic standards in absolute methanol.

Sample preparation

Heart: Samples were organically extracted as described previously [15]. In brief, samples were extracted by the addition of 3 g ammonium sulfate, 5 ml extraction solvent (50% CCl_4/50% isopropyl alcohol), brief vortexing, and shaking for 30 min at room temperature. Extracted samples were centrifuged at 2000 rpm for 15 min, and the top (organic) phase transferred to a clean 15-ml polypropylene tube and dried down under N_2 at 37°C. Then all samples were resuspended in 2 ml ammonium formate buffer (AFB) and either held overnight at –70°C or purified immediately by perfusion through Sep-Pak cartridges (Waters, Milford, Mass.) that had been equilibrated with 5 ml MeOH, 5 ml 50% MeOH/H_2O and 6 ml AFB. After the sample had drained through the cartridges, vacuum was applied, and the cartridges washed with 4 ml AFB followed by 1 ml heptane. Vacuum was removed and the samples were eluted into clean 15-ml polypropylene test tubes with 5 ml MeOH, and dried down under N_2 at 37°C. All samples were dissolved in 400 µl mobile phase under the initial conditions and stored in the dark at –20°C until HPLC analysis. The use of this two-step recovery method improved the stability of chromatograms over time. The mean recoveries from three samples (50 ng/sample) for doxorubicin, doxorubicinol and daunorubicin were 27%, 44%, and 52%, respectively. The intraassay precision (coefficient of variation in percent) in analysis of 200 ng of compound in six samples was 17% for doxorubicin, and 6% for doxorubicinol. The interassay precision for 200 ng of compound in six samples was 9% for doxorubicin and 10% for doxorubicinol.

Plasma: Standard curve samples were constructed as described above using 0.5-ml aliquots of human plasma. Rat plasma and standard curve samples (0.5 ml) were diluted to 2 ml with AFB and applied directly to equilibrated Sep-Pak cartridges and processed as previously described [15, 16] as follows. Sep-Pak extraction cartridges were conditioned with 4 ml methanol, 4 ml methanol/water (50:50, v/v), and 5 ml 0.1% AFB. Then plasma samples were diluted in 4 ml AFB and passed through the cartridges followed by 4 ml 0.1% AFB and 1 ml heptane. The cartridges were then aspirated to dryness and the samples were eluted from the cartridges with 7 ml methanol, collected and dried at 45°C. Samples, reconstituted in 0.1% AFB, were injected onto the HPLC column. Intraassay coefficients of variation for doxorubicin and doxorubicinol (20 ng/ml) were 4.1% and 4.8%, respectively. Interassay coefficients of variation for doxorubicin and doxorubicinol (20 ng/ml) were 10%.

Stability of samples: Doxorubicin, doxorubicinol, and daunorubicin were shown to be stable as stock solutions in methanol when kept in the dark at 4°C. Repeated HPLC analysis of these stock dilutions over many months showed no split or secondary peaks. On the other hand, samples not removed from their biological matrix (plasma or tissue) rapidly degraded unless handled on ice, quick-frozen, and stored at –70°C or in liquid nitrogen. Once extracted and purified, such samples were stable at –20°C for at least 30 days.

HPLC instrumentation and conditions

HPLC analysis was performed as previously described [15, 16] with minor modifications. In brief, the assay employed a Waters gradient system consisting of two Model 510 pumps, a Model 680 gradient controller and a Model 715 WISP autosampler. The method used a Luna 5-µm phenylhexyl chromatography column (4.6×150 mm) and phenylhexyl precolumns purchased from Phenomenex (Rancho Palos Verdes, Calif.). Sep-Pak C_{18} cartridges (Waters) were used for sample cleanup. The fluorescence detector was a Kratos Spectroflow 980 fitted with a xenon lamp (Photon Technology, Lawrenceville, N.J.). A Hewlett Packard 3395 integrator (Agilent, Wilmington, Del.) was used for data collection.

The phenylhexyl HPLC column fitted with a precolumn insert with the same packing was equilibrated with 76% mobile phase A (0.16 *M* AFB brought to pH 4.0 with 95% formic acid) and 24% mobile phase B (acetonitrile) at a flow rate of 2 ml/min. With the use of a linear gradient, mobile phase conditions were changed over 8 min to 70% A/30% B and maintained for 7 min. Over the next minute the gradient was changed to 10% A/90% B and maintained for 2 min. The gradient was returned to the initial conditions over 2 min and re-equilibrated for an additional 10 min. Signal detection was carried out at $\lambda_{ex}470/\lambda_{em}550$.

Data analysis

Both plasma and heart doxorubicin and doxorubicinol concentrations from rats at each time point were randomly assigned to create concentration/time profiles. Pharmacokinetics parameters were determined by noncompartmental analysis (Kinetica, InnaPhase, Philadelphia, Pa.) using a single-dose i.v. bolus drug administration model. The mean weights in the young and old rat groups were used for normalization of pharmacokinetics parameters. The data were analyzed by two-way ANOVA with age and dexrazoxane treatment as factors using Sigma Stat (Jandel Scientific software, San Rafael, Calif.). The level of statistical significance was $P < 0.05$. Data are expressed as means ± SE.

Results

Mean plasma concentrations of doxorubicin following single-dose administration of doxorubicin 2 mg kg^{-1} i.v. to young and old rats with or without pretreatment with dexrazoxane are shown in Fig. 1. The peak plasma concentration of doxorubicin in old rats at 1 h was 94% ($P < 0.001$) and the terminal plasma concentration at 72 h was 62% ($P < 0.05$) higher than the corresponding

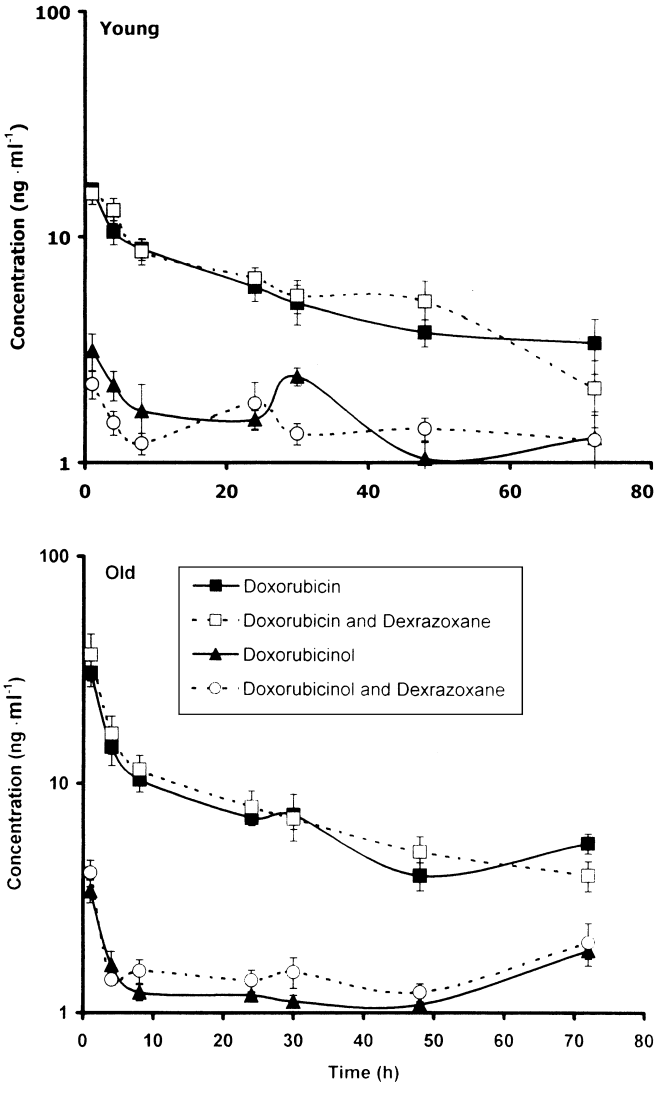

Fig. 1a, b Plasma concentrations of doxorubicin and doxorubicinol following administration of doxorubicin 2 mg kg^{-1} as a single-dose intravenous bolus administration over 1 min without (*black symbols*) and with (*white symbols*) pretreatment with dexrazoxane 50 mg kg^{-1} by intraperitoneal injection in young (**a**) and old (**b**) Fischer 344 rats. Values shown are means ± SE (1 ng ml^{-1} is equivalent to 1.72 n*M* doxorubicin and 1.85 n*M* doxorubicinol)

values in young adult rats (Table 1). Correspondingly, the area under the plasma concentration/time curve to infinity (AUC_∞) was increased by 20% in old compared with young rats (Table 1; $P < 0.01$). Although the terminal elimination half-life of doxorubicin was unaffected by age, the volume of distribution at steady-state (Vd_{ss}) was decreased in the old compared with the young adult rats (Table 1; $P < 0.05$). Systemic clearance of doxorubicin was reduced by 19% in the old rats (Table 1; $P < 0.01$).

The plasma concentrations of the alcohol metabolite, doxorubicinol, were much lower than those for the parent compound (Fig. 1). Because the drug concentrations were near the limits of detection, the slope of the

Table 1 Effect of age and dexrazoxane pretreatment on plasma doxorubicin and doxorubicinol pharmacokinetics. Data are presented as means ± SE (*n* number in each group unless indicated otherwise in parentheses)

	Age group	Treatment with/without dexrazoxane	AUC_∞ (ng h ml^{-1})	AUC_t (ng h ml^{-1})	Half-life (h)	Vd_{ss} (l kg^{-1})	Vd_{area} (l kg^{-1})	Cl (ml min^{-1} kg^{-1})	Peak concentration (ng ml^{-1})	Terminal concentration (ng ml^{-1})
Doxorubicin	Young ($n=8$)	Without	516 ± 44	352 ± 19	37 ± 7	194 ± 19	199 ± 23	68 ± 6	16 ± 1	3.4 ± 0.9
		With	460 ± 34	357 ± 30	24 ± 3	156 ± 11	149 ± 13	75 ± 6	16 ± 2	2.0 ± 0.6
	Old ($n=6$)	Without	620 ± 40*	454 ± 30*	34 ± 5	153 ± 16*	160 ± 18	55 ± 4*	31 ± 4*	5.5 ± 0.6*
		With	691 ± 74*	553 ± 39*	34 ± 4 ($n=5$)	128 ± 12*	140 ± 9 ($n=5$)	51 ± 5*	37 ± 9*	4.0 ± 0.6*
Doxorubicinol	Young ($n=8$)	Without		96 ± 9					3.1 ± 0.6	1.3 ± 0.4 ($n=6$)
		With		77 ± 12					2.2 ± 0.3	1.3 ± 0.4 ($n=5$)
	Old ($n=6$)	Without		96 ± 5					3.4 ± 0.4	1.9 ± 0.2
		With		110 ± 5					4.1 ± 0.5	2.0 ± 0.4

*$P < 0.05$, effect of age; two-way ANOVA

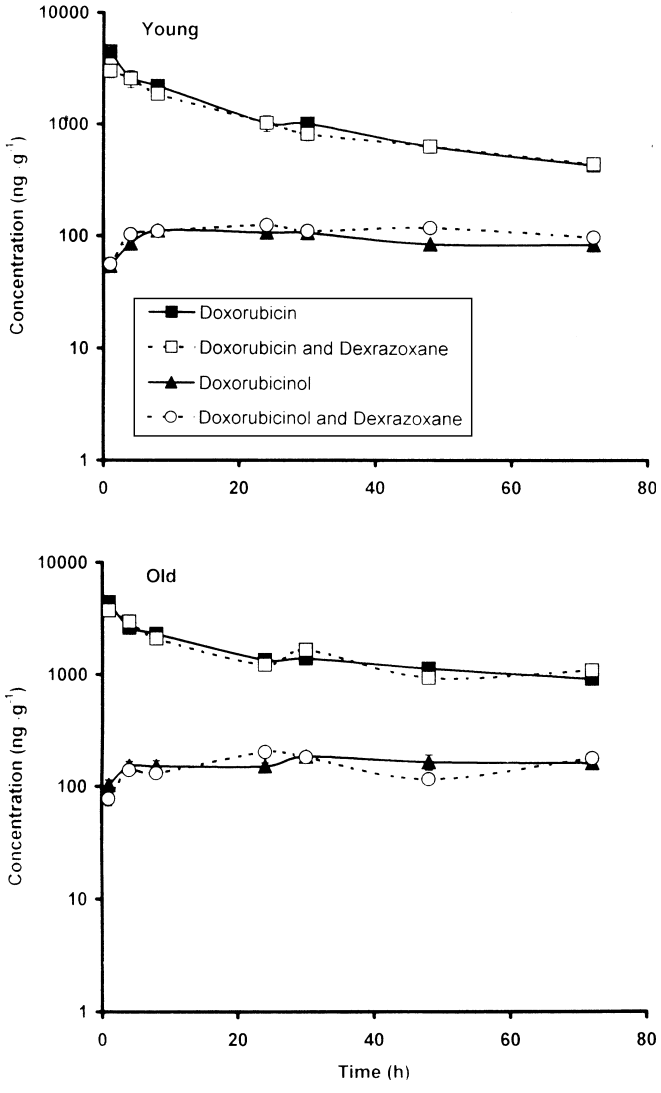

Fig. 2a, b Cardiac concentrations of doxorubicin and doxorubicinol following administration of doxorubicin 2 mg kg^{-1} as a single-dose intravenous bolus administration over 1 min without (*black symbols*) and with (*white symbols*) pretreatment with dexrazoxane 50 mg kg^{-1} by intraperitoneal injection in young (**a**) and old (**b**) Fischer 344 rats. Values shown are means ± SE (1 ng g^{-1} wet weight of tissue is equivalent to 1.72 n*M* doxorubicin and 1.85 n*M* doxorubicinol)

terminal curve could not be calculated reliably. The AUC_t (0–72 h) for doxorubicinol was unaffected by age (Table 1). Similarly neither the peak nor the terminal concentrations of doxorubicinol were affected by age (Table 1). Note that the AUC_t (0–72 h) for doxorubicin was about fourfold higher than that for doxorubicinol.

The effect of age on cardiac concentrations of doxorubicin after i.v. bolus administration of doxorubicin 2 mg kg^{-1} i.v. are shown in Fig. 2. There was a significant effect of age on doxorubicin kinetics in the heart, with a 36% increase in AUC_t (0–72 h) ($P < 0.001$), a 61% increase in AUC_∞ ($P < 0.001$), and a 44% increase in elimination half-life ($P < 0.001$) in the old compared with the young rats (Table 2). While peak concentrations were unaffected, the terminal concentrations were increased by over 100% in the old compared with young adult rats ($P < 0.001$; Table 2), likely due to the difference in elimination half-life. In addition, cardiac doxorubicinol kinetics were altered by age, with an 82% increase in the AUC_t (0–72 h) ($P < 0.001$), a 66% increase in peak concentrations ($P < 0.001$) and a 90% increase in terminal ($P < 0.001$) concentrations in the older animals (Table 2).

Dexrazoxane had little effect on the plasma concentrations of doxorubicin or doxorubicinol in either group, which were virtually superimposable on the respective anthracycline concentrations after doxorubicin administration alone (Fig. 1). The systemic plasma kinetics of doxorubicin were not altered by dexrazoxane pretreatment (Table 1). Likewise, the AUC_t (0–72 h), and peak and terminal plasma concentrations of doxorubicinol were unaffected by dexrazoxane in both age groups (Table 1). In heart, doxorubicin AUC_∞, the AUC_t (0–72 h) and the elimination half-life in heart were unaffected by dexrazoxane (Table 2). Interestingly, the peak cardiac concentrations of doxorubicin were decreased by 33% in young and 16% in old rats by dexrazoxane pretreatment ($P < 0.02$; Fig. 2; Table 2). Cardiac doxorubicinol AUC_t (0–72 h), and peak and terminal concentrations were not altered by dexrazoxane pretreatment (Table 2). The elimination half-life of doxorubicinol could not be calculated since the decay slope had not declined sufficiently by 72 h (Fig. 2).

The ratios of cardiac over plasma AUC_t (0–72 h) for doxorubicin and doxorubicinol are shown in Fig. 3 and demonstrate that the concentrations of doxorubicin in heart were dramatically (about 200-fold) higher than in plasma. Likewise, doxorubicinol concentrations in heart greatly exceeded those in plasma, albeit less so than doxorubicin. Age and dexrazoxane did not significantly alter the heart to plasma ratios of parent drug. Because the ratio of heart to plasma concentrations of doxorubicinol in the young control group was relatively low, it was significantly less than the ratio in the old control group ($P < 0.05$), and less than in the dexrazoxane-treated young group ($P < 0.05$). As a result, the effect of dexrazoxane pretreatment on doxorubicinol heart/plasma ratios differed between the age groups ($P < 0.05$).

Discussion

The beneficial effect of dexrazoxane in ameliorating chronic anthracycline cardiotoxicity has been well established in humans [8] and also in animals such as the rat [9, 10]. It currently is approved for use with anthracyclines in patients with breast cancer and has been recommended by the American Society of Clinical Oncology for patients with metastatic breast cancer who have received a cumulative doxorubicin dose 300 mg/m^2 or more and who may benefit from additional chemotherapy [17]. However, little is known about the effect of dexrazoxane on anthracycline pharmacokinetics, in

Table 2 Effect of age and dexrazoxane pretreatment on cardiac doxorubicin and doxorubicinol pharmacokinetics. Data are presented as means ± SE (*n* number in each group unless indicated otherwise in parentheses)

	Age group	Treatment with/without dexrazoxane	AUC_t ($\mu g\ h\ g^{-1}$)	AUC_∞ ($\mu g\ h\ g^{-1}$)	Half-life (h)	Peak concentration ($ng\ g^{-1}$)	Terminal concentration ($ng\ g^{-1}$)
Doxorubicin	Young ($n=8$)	Without	78 ± 2	100 ± 4	32 ± 3	4496 ± 406	427 ± 22 ($n=6$)
		With	70 ± 5	83 ± 6	25 ± 2	3011 ± 404**	438 ± 22 ($n=6$)
	Old ($n=6$)	Without	106 ± 3*	161 ± 16*	46 ± 7*	4417 ± 554	886 ± 104*
		With	104 ± 5*	151 ± 9*	42 ± 1*	3728 ± 145**	1058 ± 119*
Doxorubicinol	Young ($n=8$)	Without	6.25 ± 0.5			111 ± 9	83 ± 6 ($n=6$)
		With	6.96 ± 0.8			124 ± 20	96 ± 7 ($n=6$)
	Old ($n=6$)	Without	11.4 ± 1*			184 ± 12*	158 ± 19*
		With	10.8 ± 0.4*			202 ± 22*	174 ± 7*

*$P < 0.05$, effect of age, **$P < 0.05$ effect of dexrazoxane; two-way ANOVA

particular in the elderly. Consequently, this study was undertaken to ascertain the age-related pharmacokinetics of doxorubicin in the rat and to determine whether the effect of dexrazoxane on the pharmacokinetics of doxorubicin is age-dependent.

Prior studies have indicated that plasma and cardiac concentrations of doxorubicin are elevated in 24-month-old rats compared with 6-week-old rats [18]. However, because the young rats were developmentally immature, differences in doxorubicin disposition between the groups may have been due to ontogenesis rather than senescence per se. Thus, we compared doxorubicin pharmacokinetics in 5-month-old (young adult) rats and 22-month-old (old) rats to provide a more appropriate aging comparison. There was a clear age-related difference in doxorubicin pharmacokinetics. We observed an increase in plasma doxorubicin AUC_∞ (Table 1) and, accordingly, a reduction in systemic clearance of doxorubicin in old compared with young rats ($P < 0.01$; Table 1). Despite this reduction in clearance in the old rats, there was no prolongation in elimination half-life with age (Table 1), perhaps in part because the volume of distribution (Vd_{ss}) was lower in the older animals (Table 1). The early peak concentrations of doxorubicin in old rats were approximately twofold higher than in young animals ($P < 0.001$). This value was obtained 1 h after drug administration and likely reflected differences in early clearance and distribution. This increase in early doxorubicin concentrations in older rats is similar to our prior observations for daunorubicin [19] and is consistent with data from studies in humans showing that early-phase doxorubicin clearance is significantly reduced in older patients [6, 7]. This may explain why old age is a risk factor for cardiotoxicity, which appears to be better predicted by the height of doxorubicin plasma concentrations rather than the total area under the plasma concentration-time curve [20]. For example, most studies have shown that doxorubicin given by slow infusion is less likely to cause cardiotoxicity than similar cumulative doses given by rapid bolus injections that produce higher peak plasma concentrations [20]. The increase in peak plasma anthracycline concentrations with age thus may be a partial explanation for age-related cardiotoxicity in humans [3] and in the rat [21].

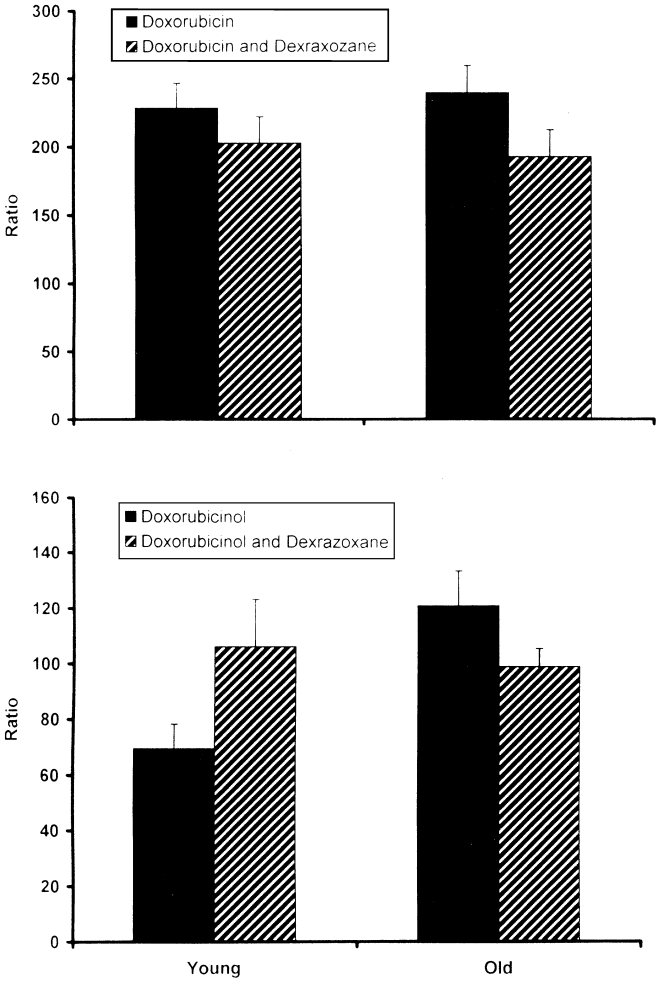

Fig. 3a, b Ratios of cardiac to plasma area under the concentration/time curves up to 72 h for doxorubicin (**a**) and doxorubicinol (**b**) following administration of doxorubicin 2 mg kg^{-1} as a single-dose intravenous bolus administration over 1 min without (*black bars*) and with (*hatched bars*) pretreatment with dexrazoxane 50 mg kg^{-1} by intraperitoneal injection in young and old Fischer 344 rats. Values shown are means ± SE

The kinetics of doxorubicin and doxorubicinol in the heart were also affected by age. Doxorubicin concentrations in the heart were increased in old rats (Fig. 2)

with a 61% increase in AUC_∞ (Table 2). The elimination half-life was increased by 44% and the terminal concentrations of doxorubicin were 107% higher in old compared to young rats. This is the first such demonstration that in the adult rat heart there is a marked increase in doxorubicin concentration with age. In addition, the cardiac doxorubicinol concentrations were higher in old than in young rats (Fig. 2) with significantly increased AUC_t (0–72 h), and peak and terminal concentrations (Table 2). Thus, cardiac levels of both the parent drug and metabolite are increased in the old rat after drug administration and could play a role in increasing the risk of anthracycline cardiotoxicity with age [21].

Doxorubicinol concentrations in plasma were low in all groups of rats with peak alcohol concentrations less than 6 ng ml^{-1} (Fig. 1). Such low plasma concentrations of doxorubicinol after doxorubicin administration in the rat have been described previously, and are consistent with the observation that doxorubicin is not a good substrate for metabolism by anthracycline reductases as has been shown in crude rat hepatic homogenates [22] or isolated perfused rat liver [23]. This contrasts with the markedly greater conversion of daunorubicin to its alcohol metabolite, daunorubicinol, in the rat, both in vivo [14, 22], and in vitro [22, 24]. Our plasma pharmacokinetics data thus confirm that systemic biotransformation of doxorubicin to doxorubicinol is limited in the rat species. In addition, age does not appear to alter this systemic conversion. This differs from our previous observations that daunorubicin is more extensively metabolized to the C-13 alcohol metabolite daunorubicinol in the aged compared to the adult Fischer 344 rat [19].

Dexrazoxane pretreatment did not significantly alter the clearance of doxorubicin, in contrast with other data [25]. In that study in Sprague-Dawley rats, plasma clearance of doxorubicin after single-dose (10 mg kg^{-1}) administration was reduced by dexrazoxane pretreatment (100 mg kg^{-1}), suggesting that dexrazoxane altered the metabolism or excretion of doxorubicin. Villani et al. [11] have also noted that cardiac doxorubicin concentrations are increased in rats pretreated with dexrazoxane following single doses of 3 mg kg^{-1} and 125 mg kg^{-1}, respectively. Rats in these studies received higher doses (100 and 125 mg kg^{-1}) of dexrazoxane than the doses used in our study (50 mg kg^{-1}) and in other experiments in the dog [26] that did not show any effect on doxorubicin kinetics. In humans, either fixed-dose [27] or incremental doses [28] of dexrazoxane have been shown to have no effect on doxorubicin pharmacokinetics, but again in doses less than those administered by Villani et al. or Vaidyanathan and Boroujerdi [11, 25]. Therefore, the effect of dexrazoxane on anthracycline disposition does not appear to occur at doses used clinically but may occur at higher doses.

The relative concentrations of doxorubicin and doxorubicinol were considerably higher in heart than in plasma (Fig. 3). The heart to plasma ratios were around 200-fold for doxorubicin and about 100-fold for doxorubicinol. The increase in cardiac doxorubicinol was unlikely to have been due to uptake but rather to metabolism from doxorubicin in cardiac myocytes. We believe this is the case since the alcohol is relatively polar and diffusion into the heart is relatively limited. Thus the ratios of heart to plasma concentrations of doxorubicinol in the present study were considerably higher than those observed by Danesi et al. following intravenous doxorubicinol administration [29]. In addition, inspection of the cardiac concentration/time curves shows that cardiac concentrations of doxorubicinol remained relatively stable while the concentrations of the parent drug were declining (Fig. 2). This suggests continued biotransformation of the parent drug to the metabolite and/or delayed elimination of the alcohol metabolite from the heart. It is important to understand the cardiac kinetics of the alcohol metabolite since it may contribute to the development of cardiotoxicity. Doxorubicinol potently inhibits cardiac sarcoplasmic reticulum Ca^{2+} transport [30, 31, 32], and is involved in destabilizing the aconitase/IRP-1 function in regulation of cytosolic iron in cardiomyocytes [33]. The former could lead to impairment of contractile function and the latter to oxidative damage by genesis of iron-dependent free radicals. Furthermore, transgenic mice, that overexpress carbonyl reductase activity in the heart, produce more cardiac alcohol metabolite after doxorubicin treatment and incur greater cardiotoxicity [34]. Conversely, chronic treatment of rabbits with C-13 deoxydoxorubicin, an analogue of doxorubicin without the carbonyl at the C-13 position, that does not form the C-13 alcohol metabolite, leads to significantly less impairment of cardiac function than doxorubicin at similar total cumulative doses [35].

Are these observations in the rat clinically relevant? Doxorubicin pharmacokinetics in the rat appears similar to those in humans, fitting a two-compartment model with a large volume of distribution and long elimination half-life [36]. The relative concentrations of the alcohol metabolite doxorubicinol that are achieved in the plasma and bile are less in the rat than in humans [37, 38]. This is consistent with the lower K_m values (0.128 ± 0.032 vs 0.275 ± 0.07 mM) for reduction of doxorubicin in rat compared to human liver [39], and the lower specific activities of doxorubicin (carbonyl) reductase (0.19 ± 0.07 vs 1.01 ± 0.19 nmol/min per mg protein at pH 8.5) in rat compared to human liver [40]. Thus, the pharmacokinetics of doxorubicin are qualitatively similar to those in humans, albeit with lower conversion to the alcohol metabolite. In this study, there was no major effect of dexrazoxane on doxorubicin pharmacokinetics, similar to observations in humans, as discussed above [27, 28]. Whether the lack of effect of senescence on the doxorubicin/dexrazoxane interaction in the rat can be extrapolated to humans is not certain. Age-related changes in drug metabolism and disposition in the rat are similar to those in humans in some but not all cases [41]. Modulation of drug disposition, for example by induction of metabolism, is impaired in old

rats [42], but the equivalent effect in older humans is variable [43]. The effect of aging on hepatic and other tissue carbonyl reductase activities that metabolize doxorubicin to doxorubicinol is not known, as is also the case for tissue P-glycoprotein, which transports anthracycline from cells, including cells involved in xenobiotic excretion. Thus, it is not possible to extrapolate the results of this study to humans, but it appears likely that, since age in the rat does not alter the effect of dexrazoxane on doxorubicin pharmacokinetics, this also is likely to be the case in humans.

This marked uptake of parent drug into the heart and conversion to the alcohol metabolite appear to be important elements in the development of cardiotoxicity [44, 45] and form the basis for therapeutic approaches to reduce the risk cardiotoxicity. For example, liposomal preparations of doxorubicin and daunorubicin putatively reduce cardiotoxicity by reducing cardiac drug uptake [46]. Phenobarbital reduces cardiotoxicity by decreasing the formation of the alcohol metabolite [47]. The present study, which demonstrated increased exposure of the aged heart to doxorubicin and doxorubicinol (Fig. 2; Table 2), suggests that such approaches to reduce cardiac doxorubicin and C-13 alcohol concentrations may help offset the risk of cardiotoxicity with advancing age.

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