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ORIGINAL ARTICLE

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Age-related pharmacokinetics of daunorubicin and daunorubicinol following intravenous bolus daunorubicin administration in the rat

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Abstract Age-related differences in pharmacokinetics may be important in determining altered anthracycline cardiotoxicity in the senescent rat and also in older humans. This study examined the effect of aging on daunorubicin pharmacokinetics in the Fischer 344 rat. Daunorubicin 7.5 mg/kg was administered i.v. to 6- and 24-month-old male Fischer 344 rats and plasma and tissue sampling was performed over 168 h for assay of daunorubicin and daunorubicinol concentrations by high-performance liquid chromatography. Systemic clearance of daunorubicin was decreased in older compared to younger animals (56 ± 4 versus 202 ± 17 ml $min^{-1} kg^{-1}$; $P < 0.05$). In addition, the area under the plasma daunorubicinol concentration/time curve was significantly increased in older rats. In the heart, the area under the concentration/time curve was significantly increased in senescence both in the case of daunorubicin (201 ± 12 versus 86 ± 4 $\mu g\,h\,g^{-1}$; $P < 0.05$) and daunorubicinol (1347 ± 118 versus 182 ± 4 $\mu g\,h\,g^{-1}$; $P < 0.05$). Furthermore, the peak mean concentrations of daunorubicin were increased in older compared to younger rats both in plasma (1078 ± 82 versus 663 ± 66 $ng\,ml^{-1}$; $P < 0.05$) and in heart (27 ± 1 versus 10 ± 1 $\mu g\,g^{-1}$; $P < 0.05$). This also was true for daunorubicinol in plasma (284 ± 39 versus 168 ± 27 $ng\,ml^{-1}$; $P < 0.05$) and in myocardium (8.6 ± 0.6 versus 2.4 ± 0.2 $\mu g\,g^{-1}$; $P < 0.05$). Following daunorubicin injection, the ratio of daunorubicinol to daunorubicin concentrations in tissues increased with time, particularly in plasma and heart in senescent rats. Thus, there are significant age-related changes in daunorubicin and daunorubicinol kinetics in the rat that could alter susceptibility to acute systemic toxicity and to chronic cardiotoxicity.

Key words Anthracyclines · Daunorubicin · Daunorubicinol · Pharmacokinetics · Rat · Aging

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Introduction

Several clinical studies indicate that the risk of cumulative, dose-dependent anthracycline cardiotoxicity is increased with age [4, 13, 30, 41]. The increased risk of anthracycline cardiotoxicity with age in these studies did not seem to be due to the presence of heart disease. For example, Palmeri et al. [30] performed a prospective study of doxorubicin cardiotoxicity using rest and exercise radionuclide angiography. In this study, multivariate analysis demonstrated that age and mid-course ejection fraction are the most significant predictors of final ejection fraction after completion of chemotherapy. Baseline ejection fraction, an indicator of underlying heart disease, does not remain significant as a predictor of a decline in ejection fraction with multivariate analysis. Preliminary studies in the rat also suggest increased cardiotoxicity with age [5, 42]. The reason for the increased risk of anthracycline cardiotoxicity with age has not been established. It could be due to pharmacodynamic factors. Anthracyclines are known to affect handling of calcium by the sarcoplasmic reticulum in myocytes [3, 8, 12, 29]. Metabolism of calcium by the sarcoplasmic reticulum is impaired with aging [14, 21], which might increase the susceptibility of the aged myocardium to toxicity from anthracyclines. Anthracyclines are quinones that undergo cyclic reduction with generation of free radicals, lipid peroxidation, and ensuing cellular damage

[17, 24]. The decline in the glutathione antioxidant pathway with age [19, 20, 37, 38] may place the senescent myocardium at increased risk of anthracycline cardiotoxicity. Pharmacokinetic factors also may play an important role in the age-related increase in cardiotoxicity. Robert et al. [32, 33] have shown a marked decline in early phase doxorubicin clearance with age. This decline in early phase clearance may influence uptake of the drug into the heart, and thus cardiotoxicity. In the rat, plasma and cardiac concentrations of doxorubicin are increased in 24-month-old compared with 6-week-old animals following single-dose doxorubicin [5]. Given the immature age of the young animals this difference in kinetics might have been due to maturation rather than senescence per se. Therefore, to avoid this limitation in this study, the pharmacokinetics of daunorubicin were compared in young adult (6-month-old) and senescent (24-month-old) Fischer 344 rats. Daunorubicinol concentrations were also compared in the two age groups, given the high concentrations of daunorubicinol that occur in plasma and tissues after daunorubicin administration in the rat [10, 27] and also the possible relevance of daunorubicinol to the development of cardiomyopathy [8].

Materials and methods

Protocol

Male Fischer 344 rats, aged 6 months and 24 months were purchased from Harlan Sprague-Dawley (Indianapolis, Ind.) and acclimatized for approximately 1 week prior to use. Rats were placed in a polythene cone restrainer and the thigh area of a hind leg treated with topical lidocaine (4%) to anesthetize the tissue. Daunorubicin (Cerubidine, Wyeth-Ayerst, Philadelphia, Pa.) 7.5 mg/kg was injected by bolus dosing into a leg vein over 1 min and the injection catheter was flushed with normal saline. Blood samples were obtained at 5, 15, 30, and 60 min and 3, 6, 24, 48, 72, 96, 120, 144, and 168 h after injection. Blood samples were collected in polypropylene tubes containing 50 units of heparin and centrifuged at 2000 rpm for 10 min to separate the plasma. Animals were sacrificed at 0.5, 3, 24, 72, 120, and 168 h after injection for recovery of tissues.

The early blood sampling up to 3 h required rapid sample collection with accurate timing. To facilitate this, animals in this sampling cohort were fitted with an indwelling intravenous i.v. 22 G silastic catheter by sterile procedure on the day prior to daunorubicin administration. This catheter was placed in the superior vena cava through the right external jugular vein and was exteriorized in the posterior cervical area. The procedure was performed using xylazine 2 mg and ketamine 20 mg intraperitoneally (i.p.) to induce anesthesia, followed by diazepam 0.5 mg i.v. for continued sedation. Animals tolerated this procedure well and were alert on the following day when daunorubicin was administered. In one cohort of animals, blood sampling was performed at 5, 15, and 30 min, followed by sacrifice. Blood samples were obtained from a second cohort of animals at 1 and 3 h, followed by sacrifice. Later blood sampling from 6 h onwards was performed in other cohorts of animals by an external jugular venipuncture with prior sedation with ketamine 40 mg i.p. From 6 h onwards a blood sample was obtained from each animal at two successive time points followed by sacrifice at the time of the second blood draw. Animals were sacrificed after anesthesia with ketamine 40 mg and xylazine 2 mg i.p.

Following sacrifice, blood was removed from the systemic circulation by perfusing the left ventricle with 0.9% NaCl and simultaneously removing blood and the perfusate through an incision in the inferior vena cava. Tissue samples (100–150 mg) were removed from the left ventricle (transmural), kidney (transverse section), liver (subcostal margin) and hip flexor skeletal muscle. Plasma and tissue samples were stored at $-70\,^{\circ}C$ until assay.

Analytical methods

The concentrations of daunorubicin and daunorubicinol in plasma were measured by high-performance liquid chromatography (HPLC) with fluorometric detection following solid-phase extraction as previously described [6, 7]. The assay of daunorubicin and daunorubicinol in tissues was performed using previously described methods [7]. Doxorubicin was employed as the internal standard. Daunorubicinol for use in standard curves was obtained by bioconversion from daunorubicin added to rat kidney homogenate in oxygenated physiological buffer as described previously [10].

Data analysis

Within each age group, plasma and tissue concentration values of daunorubicin and daunorubicinol, obtained from eight rats at each time point were randomly assigned to one of eight concentration/time profiles. Pharmacokinetic parameters were determined for each of the eight concentration/time curves within each age group. The areas of concentration/time profiles were estimated by the trapezoidal rule. The slopes of the terminal portions of the concentration/time curves were determined by exponential linear regression. Pharmacokinetic parameters were calculated by model-independent methods [15] using a Pharm-NCA software program (Simed, Créteil, France). The mean values for weight in young (368 ± 4 g) and old (427 ± 5 g) were used to normalize pharmacokinetic parameters. Parameters were compared between age groups using Student's *t*-test for unpaired data for single comparisons or ANOVA with the Student-Neuman-Keuls test for multiple comparisons using SigmaStat (Jandel Scientific, San Rafael, Calif.) or Instat (GraphPad, San Diego, Calif.) software. The level of statistical significance was $P < 0.05$ (two-tailed comparison). Data are expressed as means $\pm$ SE.

Results

Peak plasma concentrations of daunorubicin in old rats were considerably higher than in young animals during the early distribution phase (1078 ± 82 versus 663 ± 66 ng/ml, $P < 0.05$; Fig. 1). In addition, plasma concentrations during the terminal phase were significantly increased with age: the concentrations of daunorubicin reached undetectable concentrations by 48 h in young animals and by 120 h in old animals. Thus, in older animals, the area under the concentration/time curve (AUC) for daunorubicin was increased 3.5-fold (2294 ± 150 versus 655 ± 62 ng h ml^{-1}; $P < 0.05$) and the terminal elimination half-life (Table 1) was increased 3.3-fold compared with young animals. (It should be noted that the terminal slope in young rats could not be estimated with confidence because of the rapid decline in plasma concentrations below the limit of detection of 0.2 ng daunorubicin; Fig. 1.) Thus,

calculated values for the terminal slope, elimination half-life, mean residence time, volume of distribution and clearance of daunorubicin in the young animals (Table 1) should be regarded as best estimates only. However, they are likely to be very close to the real values, since the concentrations of daunorubicin or daunorubicinol after the last calculated concentrations were low (<0.2 ng/ml of drug or metabolite) and likely to have had almost negligible effects on half-life and AUC. The systemic clearance of daunorubicin was significantly reduced with age (Table 1).

The peak mean plasma concentration of daunorubicinol was significantly elevated (284 ± 39 versus 168 ± 27 ng ml^{-1}; $P < 0.05$) and the terminal plasma concentration (14 ± 2 versus 3 ± 0.3 ng ml^{-1}; $P < 0.05$) of daunorubicinol was significantly increased in old compared with young rats (Fig. 1). The total plasma daunorubicinol AUC was markedly increased with age (6991 ± 918 versus 3905 ± 177 ng h ml^{-1}; $P < 0.05$; Fig. 2), reflecting significantly increased terminal plasma concentrations in the older animals (Fig. 1).

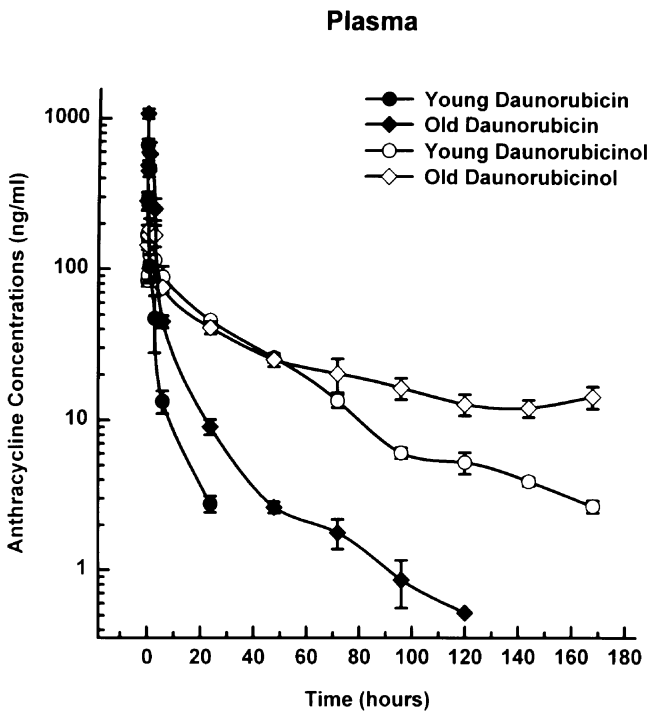

Fig. 1 Plasma concentrations of daunorubicin and daunorubicinol in young and old Fischer 344 rats following administration of daunorubicin 7.5 mg kg^{-1} by i.v. bolus. Values are shown as means ± SE

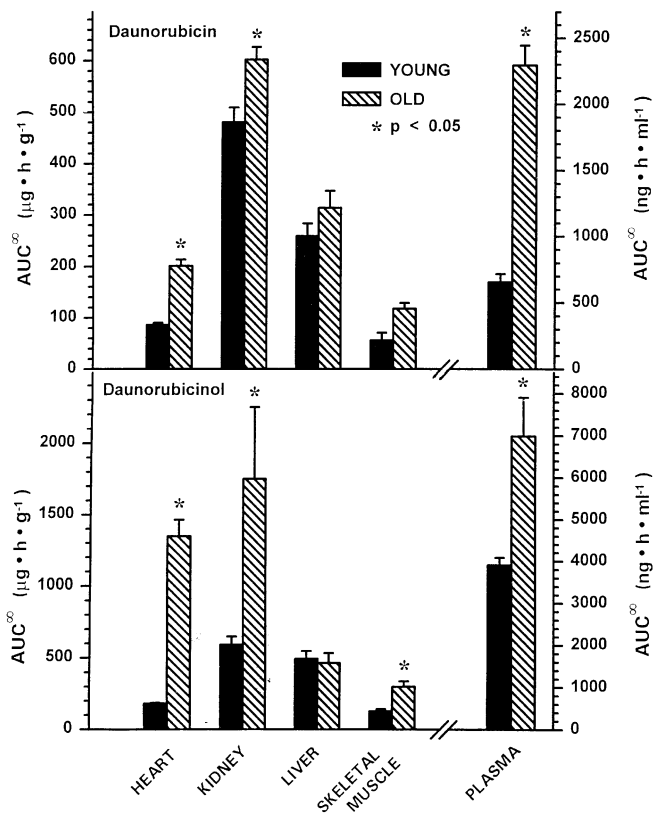

Fig. 2 Area under the curve to infinity (AUC$^{\infty}$) for daunorubicin and daunorubicinol in plasma, heart and other tissues in young and old Fischer 344 rats following administration of daunorubicin 7.5 mg kg^{-1} by i.v. bolus. *Bars* and *brackets* indicate means ± SE

Table 1 Pharmacokinetics of daunorubicin and daunorubicinol in plasma in young and old Fischer 344 rats following administration of daunorubicin 7.5 mg kg^{-1} by i.v. bolus

Group	λ (h^{-1})	$t_{1/2}$ (h)	V_{β} (l kg^{-1})	V_{ss} (l kg^{-1})	AUC_t (ng h ml^{-1})	MRT (h)	Cl (ml min^{-1} kg^{-1})
Daunorubicin							
Young ($n = 8$)	0.101	7.9	142.3	68.0	627	5.5	202
SE	0.012	1.3	28.6	11.5	63	0.7	17
Old ($n = 8$)	0.033*	25.6*	124.1	39.9	2247*	11.5*	56*
SE	0.005	4.5	23.2	9.8	153	2.4	4
Daunorubicinol							
Young ($n = 8$)	0.011	64.6			3640	52.7	
SE	0.001	4.9			162	2.5	
Old ($n = 8$)	0.007*	136.2*			4519*	162.4*	
SE	0.001	32.6			156	40.4	

* $P < 0.05$, young versus old rats

Table 2 Peak and terminal concentrations of daunorubicin and daunorubicinol in plasma and tissues. The peak concentrations represent the highest mean values that occurred in plasma and in tissues. For daunorubicin these occurred at 5 min in plasma and at 30 min in tissues. For daunorubicinol these values occurred at 5 min in plasma and at 30 min in most tissues. The terminal concentrations represent the lowest mean concentrations that occurred at 168 h in most cases. The times of other peak and terminal concentrations are shown in parentheses

Group	Heart ($\mu g\,g^{-1}$)	Kidney ($\mu g\,g^{-1}$)	Liver ($\mu g\,g^{-1}$)	Skeletal muscle ($\mu g\,g^{-1}$)	Plasma ($ng\,ml^{-1}$)
Peak concentrations					
Young rats/Daunorubicin	9.7 ± 0.5	40.7 ± 6.6	24.0 ± 1.8	4.5 ± 0.3	663 ± 66
Young rats/Daunorubicinol	2.4 ± 0.2 (3 h)	11.4 ± 2.1	61.4 ± 6.7	2.4 ± 0.4 (24 h)	168 ± 27
Old rats/Daunorubicin	27.3 ± 0.9*	33.5 ± 3.5	30.0 ± 5.5	4.2 ± 0.6	1078 ± 82*
Old rats/Daunorubicinol	8.6 ± 0.6*	8.5 ± 1.3	32.6 ± 4.7*	2.9 ± 0.5 (24 h)	284 ± 39*
Terminal concentrations					
Young rats/Daunorubicin	0.03 ± 0.01	0.19 ± 0.01	0.08 ± 0.01	0.80 ± 0.09 (24 h)	2.8 ± 0.35 (24 h)
Young rats/Daunorubicinol	0.23 ± 0.03	1.15 ± 0.38	0.11 ± 0.01	0.05 ± 0.003	2.7 ± 0.25
Old rats/Daunorubicin	0.09 ± 0.001*	0.45 ± 0.11*	0.09 ± 0.02	0.05 ± 0.0001*	0.98 ± 0.32* (96)
Old rats/Daunorubicinol	2.37 ± 0.13*	3.42 ± 0.60*	0.61 ± 0.05*	0.27 ± 0.01*	14.4 ± 2.4*

* $P < 0.05$ young versus old rats

In heart tissue, the peak concentration of daunorubicin was almost threefold higher ($P < 0.05$; Table 2, Fig. 3) and the AUC was over twofold greater in old compared with young rats ($P < 0.05$; Fig. 2). Furthermore, the elimination half-life of daunorubicin was prolonged by 47% in older rats ($P < 0.05$; Fig. 4). The age-related differences in myocardial daunorubicinol concentrations were even more marked. Peak cardiac concentrations were almost fourfold higher ($P < 0.05$; Table 2, Fig. 3) in old compared with young rat heart. In addition, terminal cardiac concentrations of daunorubicinol were severalfold higher (Table 2; Fig. 3), with marked prolongation of the apparent elimination half-lives (252 ± 29 versus 39 ± 4 h; $P < 0.05$; Fig. 4) and a threefold increase in the AUC^t for daunorubicinol in old compared with young hearts (500 ± 33 vs $168 \pm 3\ \mu g\,h\,g^{-1}$; $P < 0.05$). (The AUC^∞ for daunorubicinol is a best estimate only because AUC was large compared with the AUC^t.) Cardiac daunorubicinol concentrations significantly exceeded those for daunorubicin in both age groups; the AUC^t (168 h) for daunorubicinol was 2 times higher than for daunorubicin in young rats and 2.6 times higher than for daunorubicin in old rats.

The AUC^∞ for daunorubicin and daunorubicinol in different tissues in young and old rats are shown in Fig. 2. Daunorubicin concentrations in old animals significantly exceeded those in young rats in heart, kidney, skeletal muscle and plasma, with no age difference observed in liver tissue. Daunorubicin AUC^∞ was highest in kidney followed by liver, heart, skeletal muscle and plasma. Daunorubicinol AUC^∞ in old rats was significantly increased in heart, kidney, skeletal muscle, and plasma compared to young animals, with no age difference in liver tissue. Daunorubicinol concentrations were highest in kidney in both age groups, followed by heart in senescent animals and by liver in young animals. The daunorubicinol AUC^∞ in heart from old rats was over sevenfold higher than in young animals (1347 ± 118 versus $182 \pm 4\ \mu g\,h\,g^{-1}$; $P < 0.05$). It should also be noted that, within age groups, daunorubicinol AUC^∞ was higher than daunorubicin in all tissues (Fig. 2).

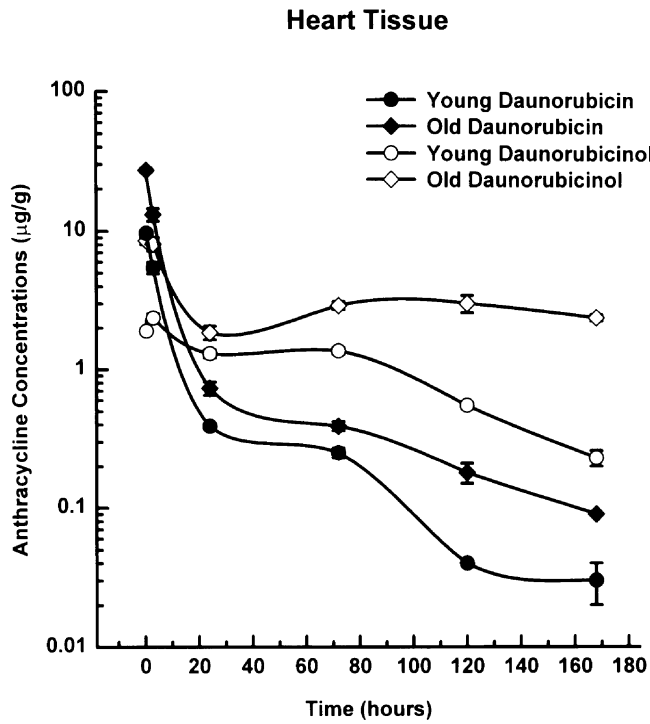

Fig. 3 Cardiac concentrations of daunorubicin and daunorubicinol in young and old Fischer 344 rats following administration of daunorubicin $7.5\ mg\,kg^{-1}$ by i.v. bolus. Values are shown as means $\pm$ SE

The elimination half-lives of daunorubicin were increased in old rats in all tissues except liver (Fig. 4). Aging had a powerful effect on the elimination half-life

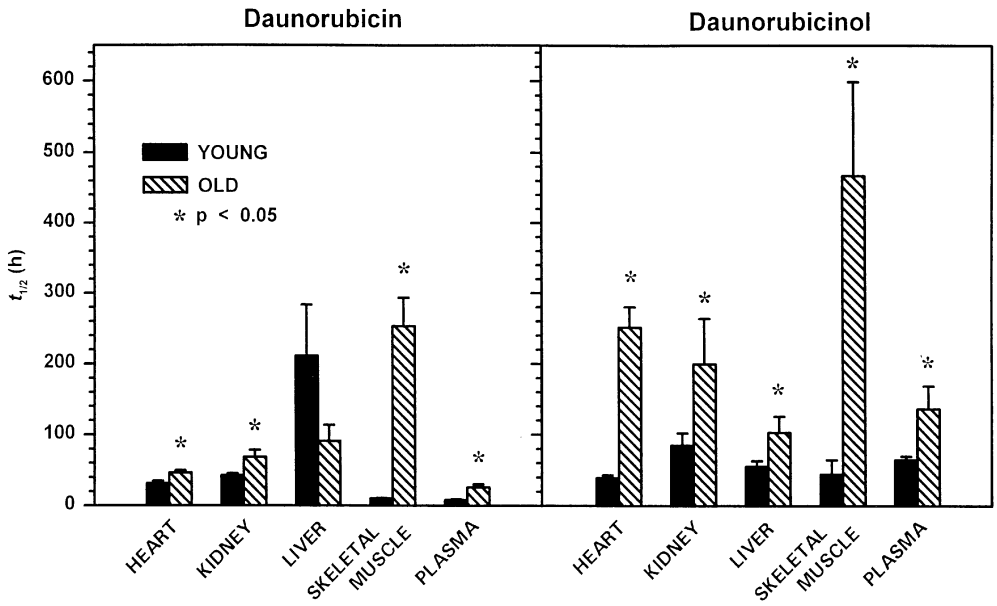

Fig. 4 Elimination half-lives of daunorubicin and daunorubicinol in plasma, heart and other tissues in young and old Fischer 344 rats following administration of daunorubicin 7.5 mg kg^{-1} by i.v. bolus. *Bars* and *brackets* indicate means ± SE

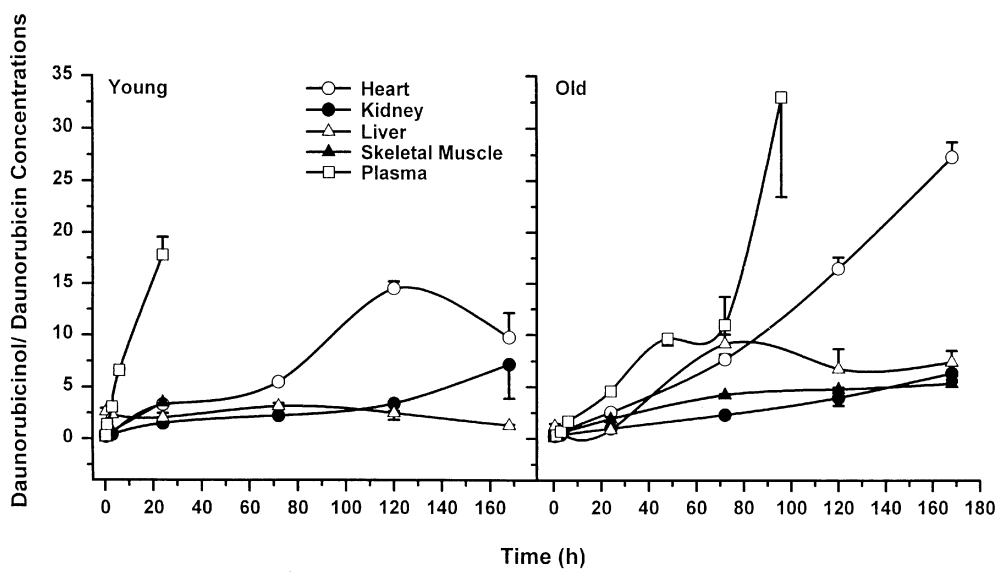

Fig. 5 Ratios of daunorubicinol to daunorubicin mean concentrations in plasma, heart and other tissues in young and old Fischer 344 rats up to 168 h following administration of daunorubicin 7.5 mg kg^{-1} by i.v. bolus. Values are shown as means ± SE

of daunorubicinol in all tissues, including plasma. In cardiac tissue, the mean half-life of daunorubicinol in old rats was over sixfold longer than in young animals (251 ± 29 versus 39 ± 4 h, respectively; $P < 0.05$).

The ratios of daunorubicinol to daunorubicin concentrations at individual sampling times in different tissues in young and old rats are shown in Fig. 5. The increases in the ratios with time were most striking in plasma and in heart, especially in old animals. Thus, in old rat heart, the ratio of daunorubicinol to daunorubicin was 27 at 168 h and 17 in plasma at 96 h (the last sampling time of measurable plasma daunorubicin concentrations in old rats). In young rats, the ratio in heart was 15 at 120 h and 8 at 168 h, while in plasma the ratio was 17 at 24 h (the last sampling time of measurable plasma daunorubicin concentrations in young rats). In other tissues, the increases in concentration ratios for daunorubicinol over daunorubicin concentrations were less striking; the maximum value in young animal tissue was 6 in kidney at 168 h and in old rat tissue was 7 in liver at 168 h.

Discussion

This study demonstrates significant age-related differences in the pharmacokinetics of daunorubicin in the

rat following i.v. bolus administration. The disposition of daunorubicin was reduced in senescent rats with an increase in the elimination half-life and the mean residence time (Table 1). The peak mean plasma concentration of daunorubicin was increased almost 2-fold ($P < 0.05$; Fig. 1, Table 2) with a 3.5-fold increase in the mean AUC ($P < 0.05$; Figs. 1, 2). In addition, the plasma kinetics of the major metabolite daunorubicinol also was altered with age; the peak mean concentration was increased by 60% ($P < 0.05$; Fig. 1, Table 2) and the elimination half-life was increased 2-fold ($P < 0.05$; Fig. 1, Table 1) in older Fischer rats. Age-related differences in pharmacokinetics also occurred in other tissues. In heart, the peak mean concentrations of daunorubicin and daunorubicinol in older rats were increased 2.8- and 3.6-fold ($P < 0.05$; Table 2, Fig. 3) and AUC^{∞} was increased 2.3- and 7-fold, respectively ($P < 0.05$; Fig. 2). The mean terminal elimination half-lives (λ) of daunorubicin and daunorubicinol in myocardium were prolonged by 47% and over 600% respectively ($P < 0.05$; Figs. 3, 4).

There are several possible explanations for the differences in daunorubicin and daunorubicinol kinetics in young and old rats. The increased plasma concentrations of daunorubicin with decreased clearance in the older rats could be due in part to altered activity of cytosolic daunorubicin reductase, with less rapid formation of daunorubicinol. Thus, in liver and kidney, two organs with considerable reductase activity, the peak concentrations of daunorubicinol, 30 min postdosing, were less in old than in young animals (significantly so in the liver; Table 2). However, these differences in peak concentrations of daunorubicinol could be related, not only to altered rate of formation, but also to altered distribution or clearance. What might explain the increase in plasma daunorubicinol concentrations in older rats? Assuming that this is related at least partly to reduced clearance of daunorubicinol, it could be inferred that the initial biotransformation of daunorubicin to deoxydaunorubicinol aglycone [16] may decline with age. Thus there may be a reduction in the activity of the hepatic, phenobarbital-inducible, NADPH-dependent, microsomal reductive glycosidase enzyme activity [1] with age. This is consistent with observations that aging is associated with a decline in hepatic microsomal metabolic enzyme activity [34, 40]. Finally, one cannot ignore the possibility that altered daunorubicin and daunorubicinol kinetics might be due to modulation with aging of the activity of the multidrug transporter P-glycoprotein; inhibition of activity of this plasma membrane efflux pump can significantly alter the AUC of the parent anthracycline and the alcohol metabolite [23].

The significant effect of aging on daunorubicin pharmacokinetics may well increase the risk of cardiotoxicity in older rats. In this study, the magnitude and duration of exposure of the myocardium to daunorubicin and also to daunorubicinol were significantly increased following single-dose administration of daunorubicin in older rats. Does anthracycline cardiotoxicity relate to the degree of drug or metabolite exposure? Several studies in humans have shown that administration of more frequent smaller doses of doxorubicin limits cardiotoxicity; weekly doses of 5–20 $mg\,m^{-2}$ cause less cardiotoxicity, with frequencies of 0–1.2% compared with rates of 1.1–13.3% in patients who receive traditional 3-weekly regimens each providing 37.5 to 60 $mg\,m^{-2}$ [2]. The incidence of cardiotoxicity remains low even in patients who received cumulative doses above 600 $mg\,m^{-2}$. Similarly, constant infusions of doxorubicin over periods of at least 12 h also reduce the risk of clinical cardiotoxicity. Legha et al. [22] found congestive heart failure in 24% of patients given doxorubicin 60 $mg\,m^{-2}$ every 3 weeks, receiving a total dose of 500–800 $mg\,m^{-2}$. In contrast, evidence of congestive heart failure was not detected in patients who were given the same cumulative dose by means of single-dose 24–48-h infusions. These alternative methods of administration (frequent low dose or by infusion) achieve lower peak plasma concentrations [2], and also presumably avoid high cardiac concentrations in humans as in the rabbit [9], although the total plasma concentration/time AUC is not altered.

In the rat, infusion of doxorubicin doses over 24 h reduces the severity of cardiomyopathy as measured by histological changes following cumulative doses of 12 $mg\,kg^{-1}$ compared with the same bolus doses [39]. Although peak plasma concentrations are increased following bolus compared to infusion administration, cardiac levels measured 24 h after the end of dosing are similar. Comparison of i.v. bolus and 24-h infusion of doxorubicin (5 $mg\,kg^{-1}$) in the rabbit has revealed that peak plasma and cardiac concentrations of doxorubicin and doxorubicinol are significantly reduced following i.v. infusion although total plasma doxorubicin and doxorubicinol AUC^{∞} are similar following both routes of administration [9]. Thus, the height of plasma and/or cardiac concentrations of anthracycline may be a predictor of cardiotoxicity.

Recent studies of cardiotoxicity following single-dose daunorubicin bolus administration support this concept [8]. In rabbits given daunorubicin 15 $mg\,kg^{-1}$ by i.v. bolus, cardiac contractility (dF/dt in the right ventricular papillary muscle) measured ex vivo 72 or 96 h after sacrifice is significantly related to peak plasma daunorubicinol concentrations ($r = -0.63$; $P < 0.05$) with a trend towards a similar relationship with peak daunorubicin concentrations ($r = -0.39$; $P = \text{NS}$). Interestingly, at the time of sacrifice, the cardiac concentrations of daunorubicinol ($r = -0.78$; $P < 0.05$), but not of daunorubicin, are significantly related to contractility (dF/dt) in the right ventricular papillary muscle. Thus, compared with young rats, each single-dose bolus administration of daunorubicin, such as in

the present study, may place old rats at higher risk of cardiotoxicity owing to significantly increased peak concentrations of daunorubicin or daunorubicinol in plasma and cardiac tissue.

Other findings also suggest the importance of conversion of parent anthracyclines to alcohol metabolites in the development of cardiotoxicity. Studies in this laboratory have shown that the cardiac concentrations of daunorubicinol, but not of daunorubicin, following a single cardiotoxic dose of daunorubicin are in the range that inhibit sarcoplasmic reticulum Ca^{2+} uptake in vitro by 40% [8]. De Jong et al. [11] studied the acute cardiotoxic effect of doxorubicin, daunorubicin, epirubicin and their 13-dihydro metabolites on mouse atrial contractile function. They observed that the 13-dihydro (alcohol) metabolites are more cardiotoxic than the corresponding parent compounds, in agreement with prior observations in this laboratory [28, 29]. Recent in vitro studies by Minotti et al. [26] suggest that reduction of the C-13 carbonyl group of the parent drug doxorubicin in the cytosol in human cardiac specimens to produce doxorubicinol is associated with redox coupling of doxorubicinol with nonferritin Fe(III)-binding proteins to yield a twofold excess of Fe(II) ions. The Fe(II) ion then becomes available to cause production of hydroxyl radicals and further oxidative stress to the myocardium. In addition, postmortem studies in patients who died at various intervals following doxorubicin treatment have shown that the cardiac concentrations of doxorubicin and doxorubicinol are similar [36]. However, doxorubicinol, but not doxorubicin, concentrations are related to the cumulative dose of doxorubicin administered. Since the cumulative dose is the major predictor of doxorubicin cardiotoxicity, perhaps the cardiac concentration of doxorubicinol achieved with long-term therapy may also relate to cardiotoxicity. In this vein, it is of interest to note that in the present study, in agreement with previous findings in this laboratory [10], the ratio of daunorubicinol to daunorubicin concentrations increased with time in cardiac tissue more than in other tissues (Fig. 5), creating the potential for a selective concentration-related toxic effect of daunorubicinol in the heart.

There is a remote possibility that the acute toxicity of daunorubicin may have affected the outcomes of this study. Many of the animals, healthy at the time of injection, appeared lethargic and anorectic in the days after injection, with decreased food intake. Acute changes in nutritional status over 7 to 10 days can modify drug metabolism in the rat [25]. Significantly different intake of food in the older compared with the younger rats could have had an effect on metabolism towards the end of the study. We did not document food intake to know whether this could have happened. It is most improbable that the differences in daunorubicin clearance, manifest within 24 h (Fig. 1), could be due to age differences in food intake. In addition, the time course of daunorubicinol plasma kinetics in the two age groups began to diverge 48 to 72 h after injection (Fig. 1). This again appears very soon for dietary effects unless abetted by infection and endotoxinemia [35] in the older animals. We consider it unlikely, therefore, that illness or dietary deprivation due to acute daunorubicin toxicity confound interpretation of the data.

Does this study imply that daunorubicin and daunorubicinol pharmacokinetics in humans also may be altered with age? There is no evidence to date that daunorubicin reductase activity is altered in humans with age. There are, however, considerable data that indicate an age-related decline in hepatic drug metabolism in humans [34], not because of altered enzyme activity but perhaps related to a reduction in hepatic mass [40]. Although the evidence regarding altered P-glycoprotein activity in immune cells in older humans is controversial [18, 31], there is at this time no information that P-glycoprotein expression or function changes with age in organs that affect drug kinetics such as liver, gut and kidney. Thus, although this study in rats does not necessarily predict that the kinetics of daunorubicin are altered in older persons, it would not be unreasonable to expect that the disposition of daunorubicin is reduced in the elderly.

This study shows that there are major differences in the pharmacokinetics of daunorubicin and daunorubicinol between young and old rats following i.v. bolus administration of daunorubicin. These differences may be of sufficient magnitude to alter the risk of chronic daunorubicin cardiotoxicity in older rats. The results underline the importance of accounting for pharmacokinetics variables in determining susceptibility to anthracycline cardiotoxicity.

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