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Daunorubicin and daunorubicinol pharmacokinetics in plasma and tissues in the rat

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Abstract Recent evidence suggests that 13-hydroxy metabolites of anthracyclines may contribute to cardiotoxicity. This study was designed to determine the pharmacokinetics of daunorubicin and the 13-hydroxy metabolite daunorubicinol in plasma and tissues, including the heart. Fisher 344 rats received 5 mg kg^{-1} daunorubicin i.v. by bolus injection. Rats were killed at selected intervals for up to 1 week after daunorubicin administration for determination of concentrations of daunorubicin and daunorubicinol in the plasma, heart, liver, kidney, lung, and skeletal muscle. Peak concentrations of daunorubicin were higher than those of daunorubicinol in the plasma (133 ± 7 versus 36 ± 2 ng ml^{-1}; $P < 0.05$), heart (15.2 ± 1.4 versus 3.4 ± 0.4 μg g^{-1}; $P < 0.05$), and other tissues. However, the apparent elimination half-life of daunorubicinol was longer than that of daunorubicin in most tissues, including the plasma (23.1 versus 14.5 h) and heart (38.5 versus 19.3 h). In addition, areas under the concentration/time curves (AUC^{∞}) obtained for daunorubicinol exceeded those found for daunorubicin in almost all tissues, with the ratios being 1.9 in plasma and 1.7 in the heart. The ratio of daunorubicinol to daunorubicin concentrations increased dramatically with time from <1 at up to 1 h to 87 at 168 h in cardiac tissue. Thus, following daunorubicin injection, cumulative exposure (AUC^{∞}) to daunorubicinol was greater than that to daunorubicin in the plasma and heart. If daunorubicinol has equivalent or greater potency than daunorubicin in causing impairment of myocardial function, it may make an important contribution to the pathogenesis of cardiotoxicity.

Key words Anthracyclines · Daunorubicin · Daunorubicinol · Pharmacokinetics · Rat · Tissue concentrations

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Introduction

Chronic, cumulative, dose-related cardiotoxicity is a major complication of anthracyclines such as doxorubicin and daunorubicin, agents that are widely used to treat hematological and solid tumors [3, 22]. Recent evidence, based on acute studies in vitro [2, 17, 18], suggests that the alcohol metabolites of these compounds may also contribute significantly to clinical cardiotoxicity. Anthracycline cardiotoxicity is thought to relate, at least in part to the concentrations of drug and/or cardiotoxic alcohol metabolite achieved in the plasma or myocardium [9, 18]. Therefore the tissue kinetics of parent anthracyclines and alcohol metabolites may provide insight into the processes that cause cardiotoxicity.

Although the pathogenesis of cardiotoxicity from anthracyclines is not firmly established, a considerable body of evidence suggests that this may be due to increased free-radical production and subsequent injury to the myocardium [10]. Other investigators consider that cardiotoxicity may be due to an effect of anthracyclines on myocardial sarcoplasmic reticulum (SR) Ca^{2+} uptake or release that occurs either directly [2, 14, 18, 19] or indirectly by an effect of free-radical production [13].

In the present study, the plasma and tissue concentrations of daunorubicin and its principal alcohol metabolite daunorubicinol were compared at various intervals for up to 1 week following a single i.v. dose of daunorubicin (5 mg kg^{-1}) in the rat, a dose comparable with single doses of 30 mg/m^2 given clinically [11].

Materials and methods

Protocol

Fisher 344 rats aged 3 months and weighing 250–300 g, obtained from a licensed supplier (Bantin & Kingman, Freemont, Calif.), were maintained for an observation period of at least 1 week on a normal diet to ensure that they were in good health. Animals were placed in a polythene restrainer and were injected with daunorubicin (Cerubidine,

Wyeth-Ayerst, Philadelphia, Pa.) 5 mg kg^{-1} over 2 min into a foreleg vein. Six animals were killed by decapitation at 30 min and at 1, 3, 6, 24, 30, 48, 54, 72, 120 and 168 h following daunorubicin injection, and blood and tissue samples were obtained. Blood samples (2–3 ml) obtained for measurement of daunorubicin and daunorubicinol concentrations were immediately centrifuged for 15 min and the decanted plasma was stored at −20° C until analysis of anthracyclines.

Blood from the systemic circulation was removed by perfusing the left ventricle with 0.9% NaCl and simultaneously removing blood and the perfusate from an incision in the inferior vena cava; the pulmonary circulation was exsanguinated by infusing saline into the right ventricle and draining through an incision in the pulmonary vein. Tissue samples (100–150 mg) from the left ventricle, kidney, liver, lung, and skeletal muscle were frozen immediately in liquid nitrogen and stored at -70° C until analysis of daunorubicin and daunorubicinol.

Analytical methods

Plasma concentrations of daunorubicin and daunorubicinol were measured following solid-phase extraction [20] by gradient high-performance liquid chromatography (HPLC) with fluorometric detection using doxorubicin as the internal standard [6–8]. Tissue samples were assayed for daunorubicin and daunorubicinol using doxorubicin as the internal standard by a method previously described elsewhere [7]. Standard curves for daunorubicin and daunorubicinol were prepared using naive rat tissue or expired human plasma.

Daunorubicinol for standard curves was obtained from homogenized kidney tissue incubated for 8 h in a bath of Krebs-bicarbonate buffer, bubbled with 95% O_2 and 5% CO_2 at 30° C, containing 5 μg ml^{-1} daunorubicin. The daunorubicinol was then extracted from the kidney tissue using $(NH_4)SO_2$ added to saturation followed by the addition of 10–20 ml 50:50 (v:v) isopropanol/chloroform. Following centrifugation for 7–10 min, the organic layer was removed, evaporated using a rotoevaporator (model RE 121, Buchi Laboratories, Flawil, Switzerland) and rinsed with methanol. The mixture was dried at room temperature using a speed vacuum evaporator (model SVC100H, Savant Instruments, Farmingdale, N.Y., USA), eluted with 3–4 ml methanol, and concentrated under nitrogen. The sample was then reconstituted in methanol. Daunorubicinol was separated by thin-layer chromatography using silica-gel 60 plates (number 5745, EM Science, Cherry Hill, N.J., USA) and mobile phase containing 89:20:14:6 (by vol.) chloroform/methanol/acetic acid/water. The daunorubicinol was scraped off the plates, washed with 15–20 ml methanol, vortexed for 3 min, and centrifuged at 3,000 rpm for 10 min. The supernatant was passed through a 0.2-μm filter, diluted in methanol, checked for purity by HPLC, and stored at 0° C until use.

At a plasma concentration of 20 ng ml^{-1}, the intraassay coefficients of variation obtained in six samples for daunorubicin and daunorubicinol were 4.1% and 4.8%, respectively, with a corresponding accuracy of within 10%. At the same plasma concentration, the respective values for interassay coefficient of variation obtained in six samples were 10% with an accuracy of within 3%. Mean values (± SE) recorded for slopes of standard curves for daunorubicin were 524 ± 68 in the heart, 441 ± 40 in the liver, 402 ± 36 in the kidney, 334 ± 72 in the lung, 652 ± 67 in the skeletal muscle, and 695 ± 13 in plasma. For daunorubicinol, the mean values recorded for slopes were 460 ± 67 in the heart, 375 ± 8 in the liver, 363 ± 15 in the kidney, 211 ± 26 in the lung, 490 ± 106 in the skeletal muscle, and 627 ± 13 in plasma. The limit of detection of daunorubicin and daunorubicinol was 0.2 ng injected directly onto the column.

Data analysis

For plasma and each tissue, concentrations of daunorubicin and daunorubicinol from six animals were combined and averaged to provide a mean value for each time point. The areas under the mean concentration/time curves were estimated by the trapezoidal rule. The slopes of the terminal portions of the mean concentration/time curves were fitted using exponential linear regression. Pharmacokinetic parameters were calculated using model-independent methods [12]. Concentrations of daunorubicin and daunorubicinol were compared using Student's *t*-test for unpaired data, with the level of significance being $P < 0.05$. Data are expressed as mean values ± SE.

Results

Following bolus i.v. injection of daunorubicin at 5 mg kg^{-1} in rats, peak plasma concentrations of daunorubicin exceeded those of daunorubicinol (Fig. 1, Table 2; $P < 0.05$). However, the concentrations of the alcohol metabolite exceeded those of the parent compound from 6 h onward, presumably because the apparent elimination half-life ($t_{1/2}$) and mean residence time (MRT) were considerably longer for daunorubicinol than for daunorubicin (Table 1). Correspondingly, the slope (λ) of the terminal elimination phase for daunorubicinol was markedly reduced as compared with that for daunorubicin. Thus, daunorubicin concentrations in plasma were undetectable after 54 h, whereas daunorubicinol concentrations were measurable for up to 168 h (0.19 ± 0.08 ng ml^{-1}) after daunorubicin administration (Fig. 1). The apparent volume of distribution (V_β), the volume of distribution at steady-state (V_{ss}), and the clearance (CL) of daunorubicinol could not be measured because the quantity of metabolite produced was not known.

The peak concentrations of daunorubicin and daunorubicinol detected in tissues significantly exceeded those measured in plasma ($P < 0.05$; Table 2). In heart tissue the peak concentration of daunorubicin was 114-fold that measured in plasma and the peak concentration of daunorubicinol was 93-fold that detected in plasma. Peak concentrations of daunorubicin were highest in the lung and kidney, followed by the liver, heart, and skeletal muscle. Daunorubicinol peak concentrations were highest in the kidney, followed by the liver, heart, lung, and skeletal muscle. Peak daunorubicin concentrations measured in all tissues and in plasma significantly exceeded those of the

Table 1 Daunorubicin and daunorubicinol plasma pharmacokinetics as determined following bolus administration of daunorubicin at 5 mg · kg^{-1} in the rat.

Drug	λ (h^{-1})	$t_{1/2}$ (h)	V_β ($l \cdot kg^{-1}$)	V_{ss} ($l \cdot kg^{-1}$)	MRT (h)	CL ($ml \cdot min^{-1} \cdot kg^{-1}$)	AUC^∞ ($ng \cdot h \cdot ml^{-1}$)
Daunorubicin	0.048	14.5	137.35	93.93	14.4	109	764
Daunorubicinol	0.030	23.1			31.9		1467

λ, elimination rate constant, $t_{1/2}$, terminal half-life, V_β, apparent volume of distribution; V_{ss}, volume of distribution at steady state; MRT, mean residence time; CL, systemic clearance; AUC^∞, area under the concentration/time curve to infinity

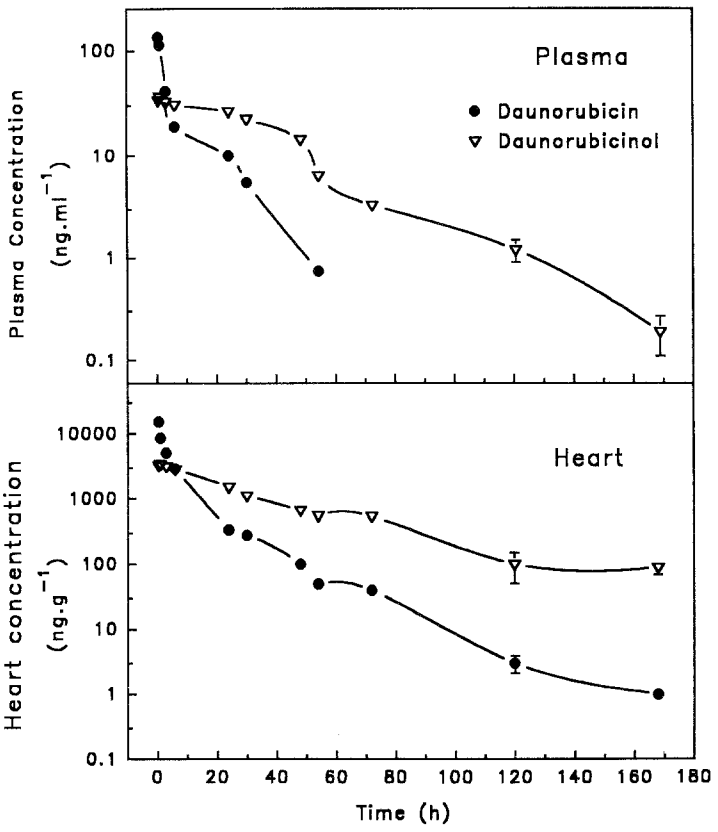

Fig. 1 Plasma (*top panel*) and cardiac (*bottom panel*) concentration/time profiles obtained for daunorubicin and daunorubicinol following i.v. bolus administration of daunorubicin at 5 mg kg^{-1} in the rat. Data are shown as mean values ± SE

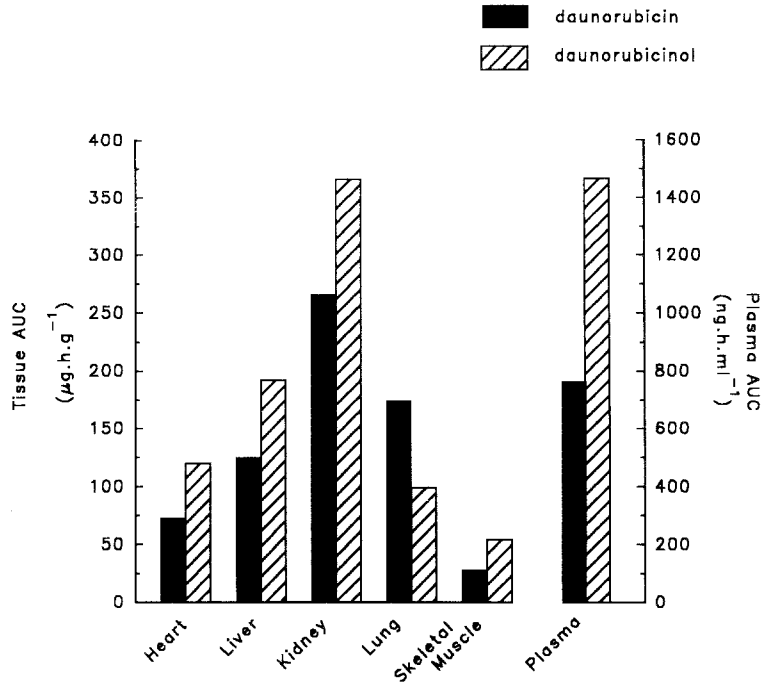

Fig. 2 AUC$^{\infty}$ values obtained for daunorubicin (*solid bars*) and daunorubicinol (*hatched bars*) in plasma and other tissues following bolus administration of daunorubicin at 5 mg kg^{-1} in the rat

alcohol metabolite. At 7 days after drug administration, however, daunorubicinol concentrations were markedly higher than daunorubicin concentrations in all tissues and in plasma ($P < 0.05$; Table 2). No daunorubicin was detectable in plasma after 54 h or in skeletal muscle after 72 h.

In tissues, the areas under the mean concentration/time curves to infinity (AUC$^{\infty}$) obtained for daunorubicin in tissues were markedly greater than those obtained in plasma (Fig. 2). The AUC$^{\infty}$ value determined for daunorubicin in heart tissue was almost 100 times the corresponding value obtained in plasma (72.6 μg h·g^{-1} versus 764 ng h ml^{-1}). The highest AUC$^{\infty}$ value for daunorubicin was achieved in the kidney, followed by the lung, liver, heart and skeletal muscle. The daunorubicinol AUC$^{\infty}$ value obtained in heart tissue (119.8 μg h g^{-1}) exceeded that found for daunorubi-

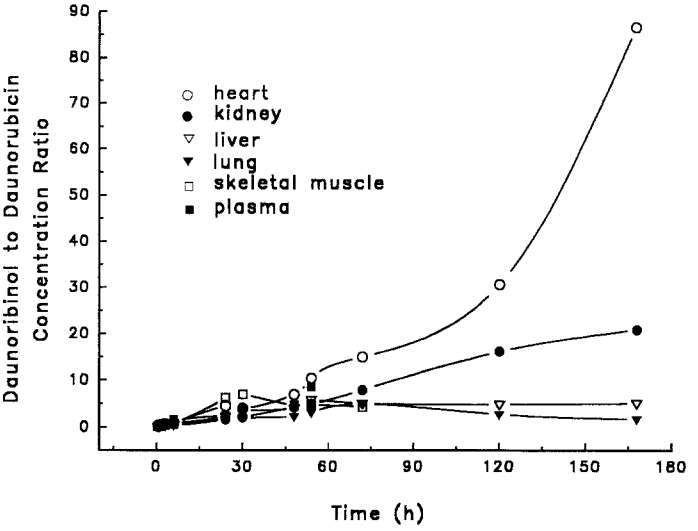

Fig. 3 Ratios of mean daunorubicinol to mean daunorubicin concentrations detected in plasma and other tissues at different time points following i.v. bolus administration of daunorubicin at 5 mg kg^{-1} in the rat

Table 2 Peak mean concentrations and mean concentrations at 168 hours of daunorubicin and daunorubicinol in plasma and tissues following i.v. bolus administration of daunorubicin 5 mg·kg^{-1} in the rat

Compound	Plasma (ng·ml^{-1})	Heart (ng·g^{-1})	Kidney (ng·g^{-1})	Liver (ng·g^{-1})	Lung (ng·g^{-1})	Skeletal Muscle (ng·g^{-1})
Peak:						
Daunorubicin	134	15220	30830	16220	41870	2680
SE	7	1350	4600	830	6610	310
Daunorubicinol	36	3390	6440	3990	2800	1270
SE	2	290	480	330	510	50
168 h:						
Daunorubicin	ND	1	14	50	40	ND
SE		0·2	1	2	2	
Daunorubicinol	0·19	90	300	230	70	20
SE	0·08	20	40	40	10	2

ND = none detected; SE = standard error of the mean

Table 3 Pharmacokinetics of daunorubicin and daunorubicinol in different tissues following i.v. bolus administration of daunorubicin 5 mg·kg^{-1} in the rat.

	Tissue	$t_{1/2}$ (h)	λ (h^{-1})
Daunorubicin			
	Plasma	14.5	0.048
	Heart	19.3	0.036
	Liver	115.5	0.006
	Kidney	22.4	0.031
	Lung	86.6	0.008
	Skeletal muscle	16.9	0.041
Daunorubicinol			
	Plasma	23.1	0.030
	Heart	38.5	0.018
	Liver	99.0	0.007
	Kidney	38.5	0.018
	Lung	38.5	0.018
	Skeletal muscle	34.7	0.020

cin and was over 80-fold that achieved in plasma (1467 ng h ml^{-1}; Fig. 2). Similar to daunorubicin, the highest AUC^{∞} value for daunorubicinol was seen in the kidney (429.7 μg h g^{-1}), with the same rank order in other tissues as that noted above for the peak tissue daunorubicinol concentrations.

The ratios of daunorubicinol to daunorubicin mean concentrations in plasma and tissues increased over time (Fig. 3), especially in the heart. In cardiac tissue the ratio was <1 for up to 6 h and increased dramatically to 87 by 168 h. Thus, from 24 to 168 h, daunorubicinol comprised from 82% to 99% of the cardiac total anthracycline, calculated as the sum of daunorubicin and daunorubicinol.

Elimination half-lives of daunorubicin in tissues were 1.3- (in the heart) to 8-fold (in the liver) longer in tissues than in plasma (Table 3). The elimination half-life of daunorubicinol was also shorter in plasma than in other tissues. The elimination half-life of daunorubicinol in plasma (23.1 h) was 60% of that in the heart (48.8 h).

Discussion

In this study we measured both daunorubicin and daunorubicinol concentrations over sufficiently long periods (up to 168 h) to permit valid determination of the pharmacokinetics of daunorubicin and daunorubicinol (substances with long elimination half-lives typically ranging from 30 to

90 h) in plasma and other tissues. We observed that the peak plasma concentrations of daunorubicin were almost 4 times higher than those of daunorubicinol (134 ± 7 versus 36 ± 2 ng ml^{-1}; $P < 0.05$; Table 2). However, the elimination half-life of daunorubicinol was prolonged as compared with that of daunorubicin (Table 1), the result being that the AUC^{∞} value obtained for daunorubicinol was 2-fold that recorded for the parent compound. Thus, although daunorubicin concentrations were not detectable in plasma after 54 h, daunorubicinol concentrations were detectable for up to 168 h after drug administration. It is possible that a further exponential phase with a longer half-life in the plasma concentration/time curve was missed because concentrations were below the limit of detection. We think that this is unlikely, however, because of the sensitivity of the assay.

The marked differences observed between tissue and plasma concentrations of daunorubicin and daunorubicinol in this study are comparable with previous data [15, 16, 21, 23] and reflect the enormous volumes of distribution of these compounds as shown for daunorubicin (Table 1). In the heart, as in other tissues, peak concentrations of daunorubicin and daunorubicinol vastly exceeded those measured in plasma (Table 2). The AUC^{∞} values found for daunorubicin and daunorubicinol in the heart were over 80-fold those seen in plasma (Fig. 2). We do not believe that the use of a 30-min sample as the first time point affected the results by underestimating the plasma AUC for daunorubicin or daunorubicinol because the portion of the AUC before 30 min is very small as compared with the total AUC values. This is due to the relatively long elimination half-lives of both compounds in plasma.

In rat myocytes in aerobic medium, uptake of daunorubicin occurred within 20 min, attaining intracellular concentrations 30- to 40-fold that measured in the medium [4]. Daunorubicin appeared tightly bound, with little leakage of drug into drug-free medium being observed. In this study, uptake into myocardium in vivo was also rapid, with peak concentrations of daunorubicin being observed at 30 min, the first sampling time (Fig. 1). Daunorubicin is a substrate for aldoketoreductases, found in many tissues, including the heart, that catalyze the conversion of daunorubicin to the 13-hydroxy metabolite daunorubicinol [1]. Although uptake of daunorubicinol from plasma cannot be ruled out, tissue metabolism of daunorubicin to daunorubicinol was likely the reason for the prolonged time to peak tissue concentration of daunorubicinol (1–24 h) as compared with daunorubicin (0.5 h).

Daunorubicinol concentrations measured after 7 days in plasma and tissues greatly exceeded daunorubicin concentrations, which mostly were below the limits of detection (Fig. 3). Daunorubicin was almost unmeasurable (1.1 ± 0.2 ng g^{-1}) at 168 h, but daunorubicinol concentrations were 90 ± 20 ng g^{-1} in the heart. Interestingly, the increase in the ratio of daunorubicinol to daunorubicin over time was uniquely prominent in the heart tissue (Fig. 3), increasing from < 1 at 6 h to 87 by 168 h. This suggests that daunorubicinol is retained to a relatively greater extent composed with daunorubicin in the heart than in other normal tissues.

Daunorubicinol causes acute cardiotoxicity in rat heart preparations in vitro [17]. Thus, the increased concentrations of daunorubicinol as compared with daunorubicin observed in the heart support the idea that the alcohol metabolite may play a role in the pathogenesis of daunorubicin-induced cardiotoxicity in vivo. Studies in isolated rat heart showing that acute cardiotoxicity following the administration of doxorubicin and other congeners was related in magnitude to the concentrations achieved in the heart preparations [5] support the relevance of cardiac anthracycline concentrations to cardiotoxicity.

Are the concentrations of daunorubicin and daunorubicinol measured in the myocardium in this study (following a dose of daunorubicin in the rat that is equivalent to clinical doses) sufficient to produce cardiotoxicity? The peak heart daunorubicinol concentrations observed in this study (3.4 ± 0.03 μg g^{-1} tissue) were in the range (10 μM, 5.4 μg g^{-1}) that inhibits rabbit sarcoplasmic reticulum (SR) Ca^{2+} uptake in vitro by almost 40% [9]. On the other hand, daunorubicin concentrations (10 μM, 5.7 μg g^{-1}) in the range of the peak concentrations found in rat myocardium in this study (15.2 ± 1.4 μg g^{-1}) had no effect on SR Ca^{2+} uptake. Assuming that the potency of daunorubicin and daunorubicinol in impairing SR Ca^{2+} is similar in the rat and the rabbit, these data suggest that daunorubicinol is more likely than daunorubicin to reach sufficient concentrations to impair cardiac function (SR Ca^{2+} uptake) in the rat following clinically relevant doses of daunorubicin. Thus, an understanding of the pharmacology and toxicology of the anthracycline metabolites is warranted.

In conclusion, daunorubicin administration in the rat yields both daunorubicin and daunorubicinol concentrations in tissues that greatly exceed plasma concentrations. Daunorubicinol concentrations achieved in the early postdosing phase are comparable with, and those attained in the late phase are considerably greater than daunorubicin concentrations obtained in various tissues, including the heart. Furthermore, daunorubicinol concentrations achieved in the heart may be sufficient to impair myocardial SR Ca^{2+} uptake significantly. These data are consistent with the premise that following clinically relevant daunorubicin doses in the rat, the hydroxy metabolite daunorubicinol may contribute significantly to the development of anthracycline cardiotoxicity.

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