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Doxorubicin and doxorubicinol pharmacokinetics and tissue concentrations following bolus injection and continuous infusion of doxorubicin in the rabbit

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Abstract. Cumulative dose-related, chronic cardiotoxicity is a serious clinical complication of anthracycline therapy. Clinical and animal studies have demonstrated that continuous infusion, compared to bolus injection of doxorubicin, decreases the risk of cardiotoxicity. Continuous infusion of doxorubicin may result in decreased cardiac tissue concentrations of anthracyclines, including the primary metabolite doxorubicinol, which may also be an important contributor to cardiotoxicity. In this study, doxorubicin and doxorubicinol plasma pharmacokinetics and tissue concentrations were compared in New Zealand white rabbits following intravenous administration of doxorubicin ($5\ mg \cdot kg^{-1}$) by bolus and continuous infusion. Blood samples were obtained over a 72-h period after doxorubicin administration to determine plasma doxorubicin and doxorubicinol concentrations. Rabbits were killed 7 days after the completion of doxorubicin administration and tissue concentrations of doxorubicin and doxorubicinol in heart, kidney, liver, and skeletal muscle were measured. In further experiments, rabbits were killed 1 h after bolus injection of doxorubicin and at the completion of a 24-h doxorubicin infusion (anticipated times of maximum heart anthracycline concentrations) to compare cardiac concentrations of doxorubicin and doxorubicinol following both methods of administration. Peak plasma concentrations of doxorubicin (1739 ± 265 vs $100 \pm 10\ ng \cdot ml^{-1}$) and doxorubicinol ($78 \pm 3$ vs $16 \pm 3\ ng \cdot ml^{-1}$) were significantly higher following bolus than infusion dosing. In addition, elimination half-life of doxorubicinol was increased following infusion. However, other plasma pharmacokinetic parameters for doxorubicin and doxorubicinol, including AUC^{∞}, were similar following both methods of doxorubicin administration. Peak left ventricular tissue concentrations of doxorubicin (16.92 ± 0.9 vs $3.59 \pm 0.72\ \mu g \cdot g^{-1}$ tissue; $P < 0.001$) and doxorubicinol (0.24 ± 0.02 vs $0.09 \pm 0.01\ \mu g \cdot g^{-1}$ tissue; $P < 0.01$) following bolus injection of doxorubicin were significantly higher than those following infusion administration. Tissue concentrations of parent drug and metabolite in bolus and infusion groups were similar 7 days after dosing. The results suggest that cardioprotection following doxorubicin infusion may be related to attenuation of the peak plasma or cardiac concentrations of doxorubicin and/or doxorubicinol.

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Introduction

Concern about cumulative dose-related, chronic cardiotoxicity of doxorubicin [6, 35] has prompted the development of various strategies to ameliorate this serious complication, including use of safer analogues [1, 18] and alteration of the mode of drug administration [7]. In cancer patients, schedule alteration, with low-dose administration every week rather than standard higher doses every 3 weeks, is associated with decreased cardiomyopathy at similar cumulative doses of doxorubicin [8, 31, 33, 36]. Low-dose anthracycline schedules also are associated with decreased cardiotoxicity in animal models [23, 27]. In further attempts to ameliorate cardiotoxicity, investigators have studied the effects of continuous infusion of doxorubicin over prolonged periods in cancer patients. Infusion over periods varying from 6 to 96 h reduces the incidence of cardiomyopathy [6, 19, 20, 26]. Increased cumulative doses of doxorubicin thus can be administered by slow, continuous infusion without increasing the risk of cardiomyopathy [6].

The mechanism remains unknown by which dose fractionation or slow infusion of anthracycline reduces cardiomyopathy. Slow infusion achieves plasma AUC (area under the plasma concentration/time curve) values of doxorubicin similar to [4, 14], or greater than [32] those following bolus dosing, but with reduced peak plasma concentrations [14]. Consequently, it has been suggested that peak plasma drug concentrations of doxorubicin may be important in predicting the relative risk of cardiotoxicity from anthracyclines.

Doxorubicinol, the principal metabolite of doxorubicin, is a potent cardiotoxic agent, inhibiting cardiac function and ATPase activities of sarcolemma, mitochondria, and sarcoplasmic reticulum [3, 22]. It is possible that reduced cardiotoxicity following dose fractionation or slow infusion is due to decreased myocardial concentrations of doxorubicin and/or doxorubicinol. This study was designed to test the hypothesis that infusion of doxorubicin produces lower cardiac tissue concentrations of doxorubicin and doxorubicinol in comparison to bolus administration in the rabbit.

Materials and methods

Protocol. Young male New Zealand white rabbits (2.7–4.2 kg), obtained from a licensed supplier, were acclimatized for 1 week and observed to ensure they were healthy prior to inclusion in the study. Intravenous catheters (1-Cath, Delmed, Canton, Mass.) were advanced about 8 in. (20.32 cm) in a marginal or central vein of each ear for doxorubicin administration and blood sampling (from the opposite ear) and kept patent with bolus administration of heparin (20 units.ml^{-1}). Doxorubicin (Adriamycin, Adria Laboratories, Columbus, Ohio) 5 mg.kg^{-1} was administered intravenously over 2 min (bolus administration; $n = 10$) or 24 h (slow infusion; $n = 10$). A constant rate infusion pump (Model 11 Infusion Pump, Harvard Apparatus, Southnantik, Mass.) infused doxorubicin at a rate calculated to deliver the total dose over a period as close as possible to 24 h (mean calculated time was 24.2 ± 0.4 h). Blood sampling (2–3 ml) was performed prior to, and at 5, 30, and 60 min and 2, 4, 7, 12, 24, 28, 32, 48, 52, 56 and 72 h after completion of bolus dosing or commencement of infusion. An equivalent volume of saline was given i. v. to replace the volume of each blood draw. Separated plasma was stored at –20° C prior to assay of doxorubicin and doxorubicinol. On the morning of day 7 (168 h) following the completion of doxorubicin administration by bolus and infusion, animals were killed by captive bolt discharge to the cranium immediately after each animal had received heparin (1000 units i. v.) to delay post-mortem coagulation. The heart was removed and the coronary circulation flushed in a retrograde fashion with ice-cold normal saline to remove blood. The dorsal aorta was flushed with saline to exsanguinate other tissues including liver, kidney, and hip flexor skeletal muscle. Tissue samples (150–200 g), obtained from the liver, kidney, hip flexor muscle, atria, left ventricle and right ventricle, were stored at –70° C until assay. In an additional series of experiments, rabbits received doxorubicin as described above and rabbits were killed 1 h after completing bolus ($n = 3$) and at completion of a 24 h infusion ($n = 3$) to compare left ventricular doxorubicin and doxorubicinol concentrations at expected times of peak tissue concentrations.

Analytical methods. Following solid-phase extraction, plasma concentrations of doxorubicin and doxorubicinol were measured by gradient HPLC with fluorometric detection as previously described [10, 11]. Frozen tissue samples (100–150 mg) were added to 2 g ammonium sulfate and 1 ml 0.9% NaCl, homogenized for 1 min, and daunorubicin was added as internal standard (5–100 µg). The homogenate was extracted using 2 ml chloroform: isopropanol (1 : 1, v : v). The mixture was vortexed for 3 min and centrifuged at 500 *g* for 12 min; the organic layer was evaporated to dryness and reconstituted in 100 µl methanol prior to HPLC analysis. Standard curves, including doxorubicin and doxorubicinol with daunorubicin as internal standard, were prepared in human plasma and matching doxorubicin-naive rabbit tissues. Doxorubicin and daunorubicin were obtained from Sigma (St. Louis, Mo.) and doxorubicinol was synthesized from doxorubicin according to Takanashi and Bachur [29] and confirmed by HPLC.

Data analysis. The slopes of the terminal portions of plasma doxorubicin and doxorubicinol concentration/time curves were fitted by linear regression, and pharmacokinetics parameters were calculated using model-independent methods [15]. Within-group or between-group parameters were compared using Student's two-tailed *t*-test for paired or unpaired data, respectively, with correction for differences in variances where appropriate. The null hypothesis was rejected at $P < 0.05$. Results are reported as mean values ± SE.

Results

Compared to bolus administration, infusion did not alter the plasma pharmacokinetics of doxorubicin (Table 1). There were no differences in the elimination rate constant (λ), the elimination half-life ($t_{1/2}$) or mean residence time (MRT) between the two modes of doxorubicin administration. The mean area under the plasma concentration/time curve to infinity (AUC^{∞}) was increased by 23% in the group that received doxorubicin by infusion compared to bolus administration, but the difference was not statistically significant. As a result, clearance of doxorubicin was not different between the bolus and infusion modes of doxorubicin administration.

The mean terminal slope of the plasma concentration/time curve (λ) for doxorubicinol was decreased by 44% ($P < 0.05$) and the mean elimination half-life ($t_{1/2}$) was prolonged by 51% ($P < 0.05$) in the doxorubicin-infused group compared to the bolus group (Table 2). Despite the prolongation of the elimination half-life of doxorubicol, there was no increase in the area under the plasma concentration/time curve to 72 h (AUC^{t}) or the AUC^{∞} for doxorubicinol following doxorubicin infusion. On the other hand, the plasma AUC^{∞} for doxorubicin was significantly greater than for doxorubicinol following doxorubicin ad-

Table 1. Comparison of doxorubicin pharmacokinetics following bolus and 24-h infusion (5 mg.kg^{-1}) in the rabbit

Group	$t_{1/2}$ (h)	λ (h^{-1})	V_{area} (l·kg^{-1})	V_{ss} (l·kg^{-1})	AUC^{∞} (ng·ml·h^{-1})	MRT (h)	CL (ng·ml·h^{-1})
Bolus (*n*: 10)							
Mean	34.9	0.021	94	66	2518	35	31.1
SE	3.3	0.002	12	9	165	3	2.4
Infusion (*n*: 10)							
Mean	29.0	0.026	69		3091	34	27.4
SE	2.9	0.002	11		272	3	2.8
P	NS	NS	NS		NS	NS	NS

$t_{1/2}$, Terminal elimination half-life; λ, terminal elimination rate constant; V_{area}, apparent volume of distribution; V_{ss}, volume of distribution at steady state; AUC^{∞}, area under the plasma concentration/time curve to infinity; MRT, mean residence time; CL, clearance. 1 ng·ml^{-1} = 1.72 nM doxorubicin

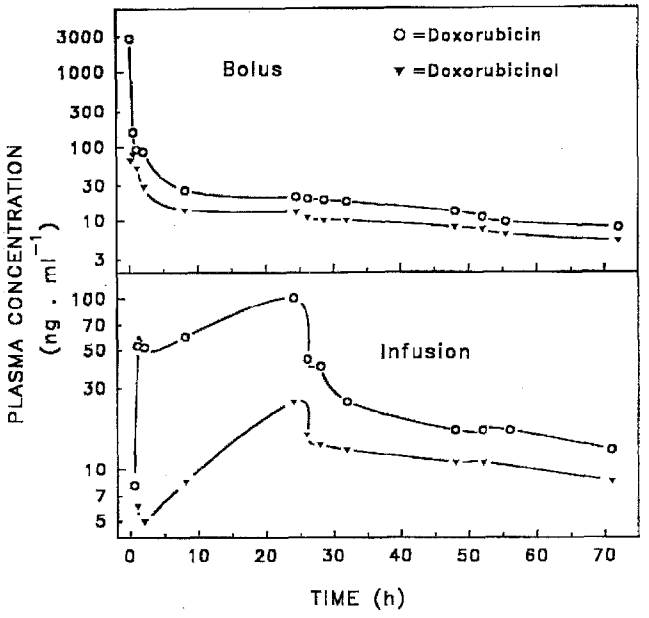

Fig. 1. Representative plasma concentration/time curve for doxorubicin and doxorubicinol following administration of doxorubicin 5 mg·kg^{-1} in the rabbit by bolus and infusion administration

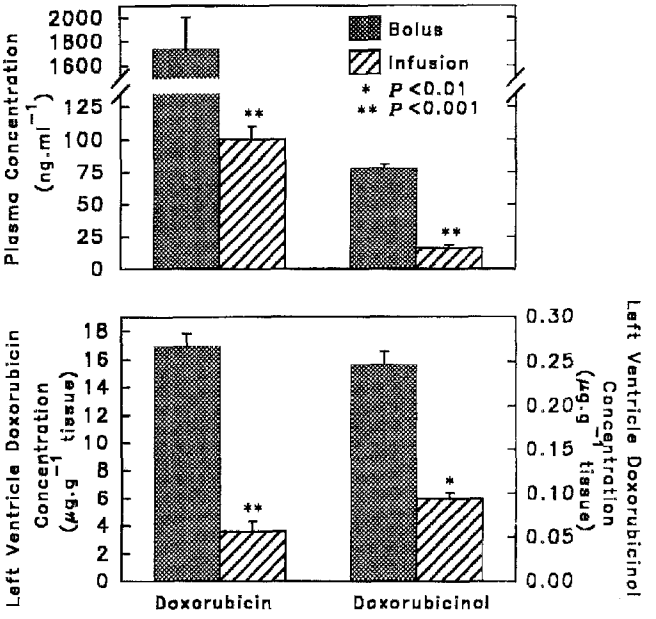

Fig. 2. Peak plasma and left ventricular concentrations of doxorubicin and doxorubicinol following bolus (2 min) and infusion (24 h) administration of doxorubicin 5 mg·kg^{-1} in the rabbit. 1 ng·ml^{-1} or 1 ng·g^{-1} = 1.72 nM doxorubicin; 1 ng·ml^{-1} or 1 ng·g^{-1} = 1.85 nM doxorubicinol

Table 2. Comparison of doxorubicinol pharmacokinetics following bolus and 24-h infusion of doxorubicin (5 mg.kg^{-1}) in the rabbit

Group	$t_{1/2}$ (h)	λ (h^{-1})	AUC^{t} (ng·ml·h^{-1})	AUC$^{\infty}$ (ng.ml.h^{-1})	MRT (h)
Bolus (*n*: 10)					
Mean	28.0	0.026	677	793	34.5
SE	2.5	0.001	67	77	3.5
Infusion (*n*: 10)					
Mean	42.2	0.018	544	837	
SE	4.8	0.002	68	134	
P	<0.05	<0.05	NS	NS	

$t_{1/2}$, Terminal elimination half-life; λ, terminal elimination rate constant; AUC^{t}, area under the plasma concentration/time curve to 72 h; AUC$^{\infty}$, area under the plasma concentration/time curve to infinity; MRT, mean residence time.
1 ng·ml^{-1} = 1.85 nM doxorubicinol

ministration either by bolus (P <0.001) or by infusion (P <0.001).

Representative plasma concentration/time curves for doxorubicin and doxorubicinol following bolus and infusion administration indicate that peak plasma concentrations of doxorubicin and doxorubicinol following bolus exceeded those after infusion (Fig. 1).

Mean peak plasma concentrations of doxorubicin (1739±265 ng·ml^{-1}) and doxorubicinol (78±3 ng·ml^{-1}) following bolus administration of doxorubicin were significantly higher than respective peak concentrations obtained following infusion (100±10 and 16±3 ng·ml^{-1}; P <0.001; Fig. 2). Doxorubicin concentrations in left ventricular tissue 1 h following bolus dosing were almost fivefold higher than concentrations obtained at the end of infusion (16.92±0.9 vs 3.59±0.72 μg·g^{-1} tissue; P <0.001). Similarly, left ventricular doxorubicinol concentrations 1 h after bolus were almost threefold higher than those obtained 24 h after beginning drug infusion (0.24±0.02 vs 0.09±0.01 μg·g^{-1} tissue; P <0.01).

There was considerable variation between tissue concentrations of doxorubicin obtained 7 days after doxorubicin administration. Cardiac concentrations were intermediate between those with high concentrations (kidney) and low concentrations (liver, skeletal muscle; Fig. 3). Following bolus and infusion of doxorubicin, cardiac concentrations of doxorubicin were similar in atrial (0.14±0.05 vs 0.15±0.7 μg·g^{-1} tissue), right ventricular (0.11±0.03 vs 0.13±0.04 μg·g^{-1} tissue), and left ventricular (0.10±0.03 vs 0.10±0.04 μg·g^{-1} tissue) samples. In other tissues, concentrations of doxorubicin also were similar following bolus and infusion of doxorubicin.

In tissues obtained 7 days after doxorubicin administration, concentrations of the primary alcohol metabolite doxorubicinol were highest in kidney, intermediate in heart and liver, and lowest in skeletal muscle (Fig. 3). Cardiac

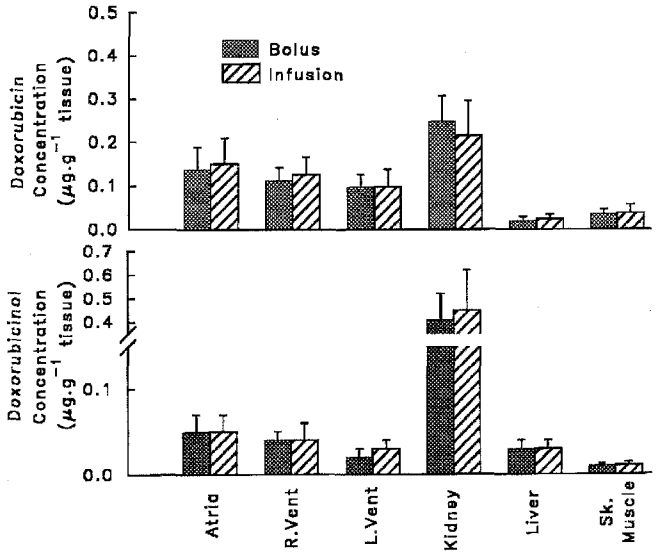

Fig. 3. Plasma and tissue concentrations of doxorubicin and doxorubicinol 7 days after completion of bolus (2 min) and infusion (24 h) administration of doxorubicin 5 mg·kg^{-1} in the rabbit. 1 ng·ml^{-1} or 1 ng·g^{-1} = 1.72 nM doxorubicin; 1 ng·ml^{-1} or 1 ng·g^{-1} = 1.85 nM doxorubicinol

concentrations of doxorubicinol were not significantly different following bolus and infusion methods of doxorubicin administration in atria (0.05 ± 0.02 vs 0.05 ± 0.02 μg·g^{-1} tissue), right ventricle (0.04 ± 0.01 vs 0.04 ± 0.02 μg·g^{-1} tissue), and left ventricle (0.02 ± 0.01 vs 0.03 ± 0.01 μg·g^{-1} tissue).

Of the total amount of drug in tissues (expressed as the sum of doxorubicin and doxorubicinol) doxorubicin comprised 70–80% in heart and skeletal muscle, similar to the proportion seen in plasma. However, the proportion of doxorubicin was considerably lower in kidney (38% post bolus, 32% post infusion) and liver (38% post bolus, 46% post infusion) with high levels of metabolite seen in the kidney and low concentrations of parent drug in the liver. The method of doxorubicin administration did not affect the relative concentrations of doxorubicin and doxorubicin in any tissue studied.

Discussion

This study was designed to determine whether doxorubicin infusion might decrease the cardiac concentrations of doxorubicin or doxorubicinol in the heart in comparison to conventional bolus dosing. The rabbit was chosen since doxorubicin pharmacokinetics are similar in the rabbit and in man [5]. Several clinical studies have demonstrated cardiac protection following infusion compared to bolus dosing of doxorubicin [16, 20, 26], raising the possibility that decreased doxorubicin and/or doxorubicinol accumulation in the myocardium may be the protective mechanism. In this study, reduced peak, rather than delayed (7 days) left ventricle concentrations of doxorubicin and doxorubicinol occurred after continuous infusion compared to bolus injection (Figs. 2 and 3). This study is in agreement with prior investigations in the rat, demonstrating that histological evidence of cardioprotection following 120 h infusion is not due to a decrease in delayed accumulation of doxorubicin in the heart [28]. In that study, tissue sampling was performed 24 h after 5 consecutive daily doses of 2 mg·kg^{-1} delivered by bolus or 24 h infusion. Cardiac doxorubicin concentrations were 2.02 ± 0.3 mg·kg^{-1} and 2.34 ± 0.33 mg.kg^{-1}, respectively. Tissue doxorubicinol concentrations were not measured, however.

The ratio of mean peak cardiac tissue concentrations of doxorubicin to doxorubicinol after bolus and infusion were 69:1 and 38:1, respectively. After 7 days the ratios were 5:1 and 3:1 respectively. Thus, cardiac concentrations of doxorubicin fell much more rapidly than doxorubicinol, suggesting that in the heart, the elimination half-life of doxorubicinol might be prolonged compared to doxorubicin. This might lead, with chronic dosing, to relative accumulation of doxorubicinol compared to doxorubicin in the myocardium, as has been observed in some chronic studies [13]. The relative accumulation of doxorubicinol compared to doxorubicin in the myocyte may be due to intracellular metabolism of the parent drug to the alcohol metabolite by aldoketoreductases. In addition, if due to simple diffusion, efflux of doxorubicinol from the myocyte may be less rapid than doxorubicin because of the greater polarity of the alcohol metabolite. The relative retention of doxorubicinol in the myocardium and greater potency to inhibit some organelle functions compared to doxorubicin [3, 22] suggests that the alcohol metabolite might be involved in the development of chronic cardiotoxicity [13].

Since the major kinetic effect of slow infusion is a decrement of peak plasma and tissue concentrations of doxorubicin and doxorubicinol, avoidance of high peaks in plasma and intracellular concentrations of doxorubicin and doxorubicinol may reduce the risk of chronic cardiotoxicity. This rationale was offered as an explanation for cardioprotection in mice treated with doxorubicin entrapped in cardiolipin liposomes [25]. In that study, animals receiving drug enclosed in liposomes had lower peak cardiac concentrations of doxorubicin and exhibited less cardiotoxicity.

Might reduction of plasma concentrations of doxorubicin and doxorubicinol be important in lowering the risk of cardiotoxicity? Anthracycline-mediated toxicity to important cationic sarcolemmal transport systems such as (Na+K)-ATPase [3, 22] may be alleviated by reducing peak plasma concentrations of doxorubicin and doxorubicinol following continuous infusion or dose fractionation of anthracycline. Inhibition of sarcolemmal enzyme systems by anthracyclines in vitro occurs at comparatively high concentrations, with doxorubicinol being considerably more potent than doxorubicin in causing toxicity (doxorubicin $IC_{50} = 400$ μg·ml^{-1}; doxorubicinol $IC_{50} = 5.4$ μg·ml^{-1} [3, 22]). Given that peak plasma concentrations of doxorubicin and doxorubicinol observed in this study (Fig. 2) are considerably lower than those required to inhibit ATPase activity in vitro [3, 22], it appears unlikely that plasma doxorubicin and doxorubicinol concentrations are sufficiently high to contribute to cardiotoxicity.

Could decreased cellular uptake of doxorubicin or doxorubicinol following infusion of doxorubicin contribute to the altered risk of cardiotoxicity? Cytofluorescence

studies indicate that cellular anthracycline uptake is not homogeneous, but is located mainly in the nucleus [12]. Tissue binding is variable and correlates closely with tissue DNA content in the rat and the rabbit [30]. Because intracellular doxorubicin and doxorubicinol distribution is not homogeneous, it is difficult to relate total tissue concentrations, as seen in these experiments with in vitro concentrations known to affect cell organelle function (IC_{50} for SR Ca-ATPase inhibition is 2 $\mu g \cdot ml^{-1}$ for doxorubicinol and >400 $\mu g \cdot ml^{-1}$ for doxorubicin) or free radical generation, two widely considered mechanisms of cardiotoxicity [21]. It could be that following bolus administration, the intracellular doxorubicin and doxorubicinol concentrations available to affect these functions directly are transiently elevated prior to nuclear uptake and binding. Thus, constant infusion may alter cardiotoxicity by decreasing intracellular concentrations of doxorubicin or doxorubicinol below those required to cause organelle toxicity. Doxorubicin also inhibits gene expression of contractile elements, including α-actin, troponin I and myosin light chain 2 [17, 24]. Whether infusion of doxorubicin attenuates the inhibition of gene expression compared to bolus injection remains unanswered.

Aside from a significant effect on the peak concentrations of doxorubicin and doxorubicinol in plasma and left ventricle (Figs. 1 and 2), drug infusion had little effect on the pharmacokinetics of the parent drug or metabolite (Tables 1 and 2). There was an increase in the elimination half-life with a corresponding decrease in the elimination rate constant for doxorubicinol following drug infusion. The reason for the difference cannot be determined, but could be due to altered rate of appearance, distribution, or elimination of doxorubicinol. Doxorubicin infusion did not alter the proportion of doxorubicin and doxorubicinol in plasma so that the AUC^{∞} for doxorubicin expressed as a percentage of the total AUC^{∞} for drug and alcohol metabolite was similar following bolus and infusion (76% vs 78%, respectively). This ratio of doxorubicin to total anthracycline (doxorubicin plus doxorubicinol) concentration in plasma was reflected in tissues obtained after 1 week in heart (bolus 80%; infusion 80%) and skeletal muscle (bolus 78%; infusion 78%; Fig. 3). In liver (bolus 38%; infusion 46%) and kidney (bolus 38%, infusion 32%), however, the proportionate concentration of doxorubicin was much decreased following either method of administration. Thus, the proportion of doxorubicin to doxorubicinol in the plasma was predictive of that in heart and skeletal muscle, but not in liver or kidney.

The high renal concentrations of doxorubicin and doxorubicinol, as seen in this study, are also seen in the rat [9] and are associated with nephrotoxicity [34]. The high concentration of doxorubicinol in the kidney is unlikely due to increased uptake of alcohol relative to parent drug into renal cells since plasma concentrations of doxorubicin exceeded those of doxorubicinol and, furthermore, doxorubicinol is more polar than the parent compound. One would expect, on the basis of plasma concentration and polarity, that doxorubicinol uptake would be less than doxorubicin from plasma into renal tissue, as seen in heart [12]. High activity of renal aldoketoreductases [2], which catalyze the conversion of doxorubicin to doxorubicinol, likely contributed to the increased concentrations of doxorubicinol in the kidney.

Although doxorubicin infusion did not alter standard pharmacokinetic parameters in comparison to bolus administration, peak plasma concentrations of doxorubicin and doxorubicinol were decreased following infusion. There was, similarly, a decrease in peak tissue doxorubicin and doxorubicinol concentrations. These observations suggest that the reduced risk of cardiotoxicity following infusion may be related to prevention of the high plasma or cardiac drug and/or metabolite concentrations incurred by bolus doxorubicin injection. Since slow infusion of doxorubicin does not prevent cardiotoxicity totally, however [6, 19, 20, 26], factors other than peak plasma doxorubicin and doxorubicinol concentrations are important in the pathogenesis of this condition.

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