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Influence of the cardioprotective agent dexrazoxane on doxorubicin pharmacokinetics in the dog

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Summary. The influence of dexrazoxane on doxorubicin pharmacokinetics was investigated in four dogs using the two treatment sequences of saline/doxorubicin or dexrazoxane/doxorubicin. Intravenous doses of 1.5 mg/kg doxorubicin and 30 mg/kg (the 20-fold multiple) dexrazoxane were given separately, with doxorubicin being injected within 1 min of the dexrazoxane dose. Both doxorubicin and its 13-dihydro metabolite doxorubicinol were quantified in plasma and urine using a validated high-performance liquid chromatographic (HPLC) fluorescence assay. The doxorubicin plasma concentration versus time data were adequately fit by a three-compartment model. The mean half-lives calculated for the fast and slow distributive and terminal elimination phases in the saline/doxorubicin group were 3.0 ± 0.5 and 32.2 ± 12.8 min and 30.0 ± 4.0 h, respectively. The model-predicted plasma concentrations were virtually identical for the saline and dexrazoxane treatment groups. Analysis of variance of the area under the plasma concentration-time curve ($AUC_{0-\infty}$), terminal elimination rate (λ_z), systemic clearance (CL_s), and renal clearance (CL_r) for the parent drug showed no statistically significant difference ($P > 0.05$) between the two treatments. Furthermore, the doxorubicinol plasma AUC_{0-t} value and the doxorubicinol-to-doxorubicin AUC_{0-t} ratio showed no significant difference, demonstrating that dexrazoxane had no effect on the metabolic capacity for formation of the 13-dihydro metabolite. The total urinary excretion measured as parent drug plus doxorubicinol and the metabolite-to-parent ratio in urine were also unaffected by the presence of dexrazoxane. The myelosuppressive effects of doxorubicin as determined by WBC monitoring revealed no apparent difference between the two treatments. In conclusion, these results show that drug exposure was similar for the two treatment arms. No kinetic interaction with dexrazoxane suggests that its coadministration is unlikely to modify the safety and/or efficacy of doxorubicin.

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Introduction

Adriamycin (doxorubicin HCl) is a potent antineoplastic agent used extensively in standard chemotherapy for breast, lung, and gastric cancer as well as sarcomas, lymphomas, and other malignancies [5]. In current practice, doxorubicin therapy is often discontinued at a cumulative dose of approximately 500 mg/m^2 due to dose-dependent cardiotoxicity [25, 29]. This limits the chemotherapeutic application of this agent, depriving responding patients of the benefits it provides. Furthermore, patients with preexisting heart disease may not be offered the drug.

Although the mechanism of doxorubicin-induced cardiotoxicity has not been clearly established, this effect may be mediated by free radical production and resultant lipid peroxidation. Iron appears to catalyze this reaction, as iron chelators have been shown to inhibit lipid peroxidation selectively and significantly [7, 22]. Dexrazoxane (ADR-529, ICRF-187), a potent chelating agent, has been shown to reduce the incidence and severity of heart-degenerative lesions associated with chronic administration of doxorubicin in a variety of animal models [15, 16, 24] and in humans [27]. Investigations in mice have shown that maximal protection is afforded when ADR-529 is given no earlier than 30 min prior to or 15 min after the administration of doxorubicin [10]. Combining these two agents provides a setting for potential drug-drug interactions that may result in an increase or a decrease in the efficacy and/or toxicity of doxorubicin. Thus far, the pharmacokinetic profiles of dexrazoxane obtained in animal species and humans have provided no evidence that interactions resulting from competitive protein binding [3, 4, 8] or direct competition for common metabolic pathways [1, 14, 28] should be expected. Unlike doxorubicin, dexrazoxane has a relatively small steady-state distribution volume (<1.0 l/kg) and a short half-life (≤ 3.0 h) and is predominantly eliminated via renal excretion [3, 8, 17]. However, doxorubicin is cleared largely through hepatic biotransformation by reduction of the side-chain carbonyl function via ubiquitous cytoplasmic reduced nicotinamide adenine dinucleotide phosphate (NADPH)-requiring aldo-keto re-

ductases. This reaction is an important metabolic pathway that produces the predominant metabolite doxorubicinol in man [28]. A reaction of lesser importance, reductive cleavage of the daunosamine sugar, is mediated by a microsomal, NADPH-dependent cytochrome P-450 reductase [1]. The dependency of these two metabolic reactions on NADPH, the formation of which is facilitated by the presence of the trace metal Mg(II) itself, suggests a potential interaction with chelators. The present pharmacokinetics study was undertaken to investigate this potential drug-drug interaction, which might alter the disposition and, thus, the therapeutic index of this important antineoplastic drug.

Materials and methods

Drugs. Dexrazoxane was supplied as a solution (lot number 87H27FY) and doxorubicin HCl was provided as a rapid-dissolution formulation (Adriamycin RDF, lot number 88A74FY). Both drugs were obtained from Adria Laboratories stock.

Animals. Four female beagle dogs (LRE, Kalamazoo, Mich.) weighing 10–13 kg were used in the study. Water was permitted ad libitum throughout, whereas food (Purina Dog Chow) was withheld for 18 h prior to and up to 4 h after dosing. Prior to and during the study, animals were housed in a United States Department of Agriculture (USDA)-approved vivarium under the auspices of an institutional animal-care committee as required by the Animal Welfare Act of 1985.

Preparation and administration of drug solutions. Adriamycin RDF was dissolved by introducing 25 ml sodium chloride for injection (Travenol Laboratories, Deerfield, Ill.) into a vial containing 50 mg active drug substance. Dexrazoxane was provided as a solution containing 10 mg free base/ml. Four female beagle dogs were randomized to the two treatment sequences of saline/doxorubicin or dexrazoxane/doxorubicin, with a 3-week recovery period elapsing between treatments. Dogs received a volume of 3 ml/kg of either sterile saline or dexrazoxane in saline [30 mg/kg; i.e., a 20:1 (v/v) dexrazoxane/doxorubicin ratio] delivered over 1 min via an E–Z Set catheter (Deseret Medical Inc., Sandy, Utah) implanted in the right cephalic vein. Within 1 min of saline (control) or dexrazoxane administration, animals were given a 1.5-mg/kg bolus dose of doxorubicin in a volume of 0.75 ml/kg via the same catheter.

Sampling and storage of biofluids. A 5-ml sample of blood was drawn from the jugular vein of each dog at time zero (predose) and at 5, 10, 15, 30, and 45 min and 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, and 120 h after the doxorubicin dose. Blood was dispensed into 10-ml heparinized vacutainers (Becton-Dickinson, Rutherford, N. J.) and mixed, and plasma was removed. Using silanized [10% (v/v) dimehthyldichlorosilane in toluene] Pasteur pipets, plasma was transferred to polypropylene test tubes for storage. Samples were protected from light throughout sample handling. Blood was also taken for a white blood cell count (WBC) at 4 days prior to and just before dosing (t_0) as well as on days 1–5, 7, and 14. Urine was collected from metabolism cages in 1-l polypropylene containers packed in dry ice. Vessels were removed every 12 h during the 1st day and then at 24-h intervals up to and including day 5. For each collection period, the total urinary volume was measured and a 5-ml aliquot was transferred to polypropylene tubes for assay at a later time. All plasma and urine samples were stored in a freezer maintained at −20° C.

Bioanalysis. Assay of doxorubicin and doxorubicinol in plasma and urine was performed using validated analytical methodology [2]. All stock or working stock solutions were prepared in silanized [10% (v/v) dimethyldichlorosilane in toluene] amber glass volumetrics to inhibit drug binding and photodegradation, respectively. Analytes were dissolved and diluted with 10 mM hydrochloric acid and then stored at 5° C. For each day's run, a six-point calibration curve (3.125, 6.25, 12.5, 25, 50, and 100 ng/ml) and controls at three levels (12, 80, and 2,000 ng/ml) were prepared in plasma or a seven point curve (25, 35, 50, 75, 100, 150, and 200 ng/ml) and controls at three levels (30, 75, and 150 ng/ml) were prepared in urine.

Plasma-sample preparation. In all, 20 µl of the internal standard daunorubicin (100 ng) was added to plasma standards and samples, followed by the sequential addition of 1.5 ml distilled water, 1.0 ml 10 mM tetrabutyl ammonium bromide, and 1 ml 1.0 M potassium phosphate buffer (pH 8.0). The solution was mixed and then poured into a 5-ml plastic syringe fixed to a Sep-Pak C18 cartridge (Waters Chromatography Division, Milford, Mass.) that had been conditioned with ~3 ml methanol followed by 3 ml of a 3:1 solution of 10 mM potassium phosphate buffer (pH 8.0): methanol. The diluted plasma was drawn through the cartridge using a vacuum of <127 mmHg. The column was washed with 3 ml water: methanol (3:1, v/v) and analytes were eluted with 3 ml 26 mM phosphoric acid in methanol. The volume of eluate was reduced to ~0.4 ml in a Buchler vortex evaporator using a vacuum of ~635 mmHg at 30° C and was then dispensed into a 1.5-ml polypropylene centrifuge tube. The sample was clarified by spinning at 15,000 rpm in a tabletop Eppendorf centrifuge for 3 min. Approximately 200 µl was transferred to a silanized Varian autosampler glass insert, and 50 µl was injected into the chromatograph.

Urine-sample preparation. In all, 60 µl internal standard (300 ng daunorubicin) was added to 1-ml urine standards and samples. Samples were acidified with 2 ml 33 mM phosphoric acid, mixed, and clarified in the Eppendorf centrifuge as described above. Approximately 200 µl was transferred to a silanized autosampler glass insert, and 50 µl was injected into the chromatograph.

Chromatographic system and separation. The chromatographic system consisted of a model III LDC Constametric pump (Riviera Beach, Fla.), an autosampler (Varian, Sunnyvale, Calif.) fitted with a Rheodyne injector, a Farrand System 3 fluorescence detector (Valhalla, N. Y.), and a Hewlett Packard 3396A integrator. The chromatographic separation was performed on a 5-µm, 25 cm × 4.6 mm Chromax C18 column (Munhall Co., Worthington, Ohio) equipped with a Brownlee (Santa Clara, Calif.) RP-18 NewGuard precolumn (32 × 1.5 mm). The mobile phase comprised acetonitrile/20 mM monobasic potassium phosphate (pH 3.0) at a ratio of 33:67 (v/v) for plasma and 31/69 (v/v) for urine samples. At a flow rate of 1.5 ml/min, the column effluent was monitored by fluorescence detection [excitation wavelength, 469 nm (monochrometer); emission wavelength, 575 nm] using a cutoff filter. The peak height ratio of analyte to internal standard was used for conversion of the detector response to concentration estimates.

Pharmacokinetic analysis. Plasma concentration versus time data were graphically evaluated to develop a working model. An empirical three-compartmental exponential decay model was selected on the basis of an evaluation of the parameter estimation error, the distribution of residuals, and the correlation coefficient. The coefficients (A_1, A_2, A_3) and exponents (λ_1, λ_2, λ_z) of the exponential terms were estimated using the PCNONLIN computer program [21]. The reciprocal of the observation was used as the weighting function. The PCNONLIN estimates, such as $AUC_{0-\infty}$ and terminal disposition rate (λ_z), were used in standard kinetic equations relating these to the dose and amount excreted in urine (X_u) to derive systemic (CL_s) and renal (CL_r) clearances and the apparent volume of distribution (Vd,λ_z) [11].

Statistical analysis. Nonparametric procedures (Wilcoxon-rank-sum test) using a model for a two-period crossover design (SAS software, SAS User's Guide, Statistical Analysis System Institute, N. C.) were employed for statistical analysis of these data. The significance level was $\alpha = 0.05$. Based on the expected variance, an n value of 4 provided better than 60% power to detect this difference in most kinetic parameters. To evaluate treatment effects, kinetic parameters for the parent drug, e.g.,

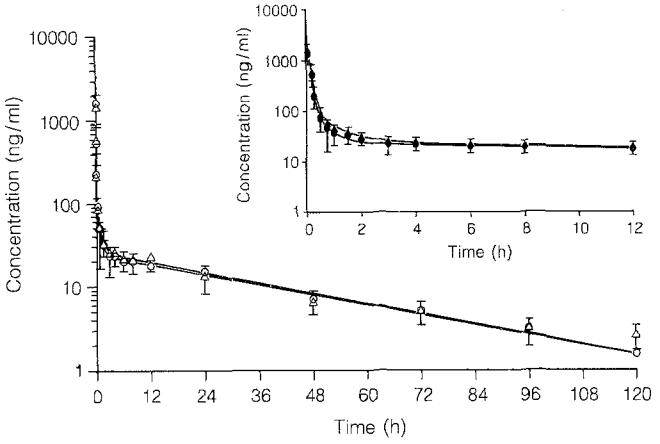

Fig. 1. Mean plasma concentration of doxorubicin in dogs given saline or dexrazoxane just prior to a 1.5-mg/kg i. v. dose of doxorubicin ($n = 4$). △, Saline/doxorubicin; ○, dexrazoxane/doxorubicin

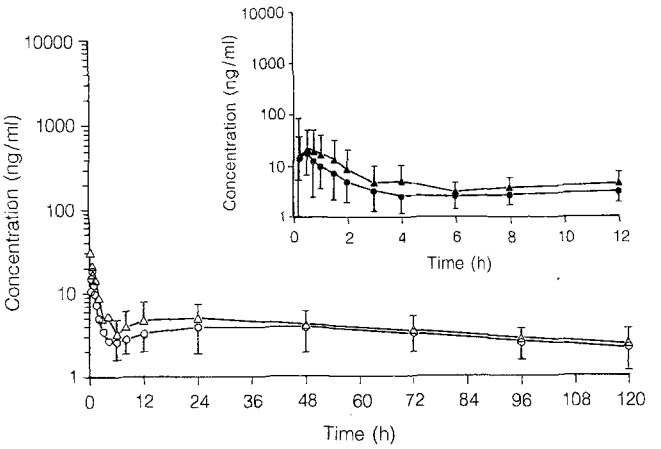

Fig. 2. Mean plasma concentration of doxorubicinol in dogs given saline or dexrazoxane just prior to a 1.5-mg/kg i. v. dose of doxorubicin ($n = 4$). △, Saline/doxorubicin; ○, dexrazoxane/doxorubicin

$AUC_{0-\infty}$, λ_z, CL_s, and CL_r were compared. The plasma AUC_{0-t} metabolite-to-parent (M/P) ratio, total urinary excretion (P+M, 0–120 h), and M/P ratio in urine (0–120 h) were also compared.

Results

Bioanalysis

The plasma assay encompassed the range of 3–100 ng/ml, and its specificity and sensitivity were excellent due to fluorescence detection. No interfering components were seen in blank plasma extracts, and the signal-to-noise ratio observed at 3 ng/ml was typically >6 and >17 for doxorubicin and doxorubicinol, respectively. Absolute recovery of analytes was >90%. The interday imprecision (%RSD) and accuracy (%bias) estimates were <11% as based on calibration standards and <19% as based on analysis of control samples. The urine assay was linear between 25 and 200 ng/ml, and the interday imprecision and accuracy were typically <6% and <9%, respectively.

Plasma clearance of doxorubicin and doxorubicinol

Due to the long elimination half-life reported for doxorubicin, an extended sampling strategy (120 h) was used to ensure adequate assessment of the terminal elimination. The ratio of $AUC_{0-t}/AUC_{0-\infty}$ indicated that 95.0% ± 2.2% of the total area was accounted for by this sampling strategy. Mean plasma concentrations of doxorubicin plotted as a function of time in the absence and presence of dexrazoxane are shown in Fig. 1. The model parameter-estimation error, the correlation coefficient, and visual inspection of weighted residual plots suggested that the plasma concentration versus time data for doxorubicin were best described by the sum of three exponentials reflecting a three-compartment open model. The mean half-life estimates for the fast and slow distributive and the terminal elimination phases in control animals (saline/doxorubicin) were 3.0 ± 0.5 and 32.2 ± 12.8 min and 30.0 ± 4.0 h, respectively. A high systemic clearance (17.2 ± 3.7 ml min^{-1} kg^{-1}) and a large distribution volume (44.4 ± 10.0 l/kg) were observed, which is characteristic for doxorubicin. Kinetic parameter estimates for both treatments are listed in Table 1. Analysis of variance (ANOVA) of the pharmacokinetic parameters λ_z, $AUC_{0-\infty}$, CL_s, and CL_r revealed no significant difference ($P > 0.05$) between the two treatment groups.

Plasma concentrations of the 13-dihydro metabolite are shown in Fig. 2. Mean systemic levels of doxorubicinol ranged from 30.7 to 2.4 ng/ml following the administration of saline and doxorubicin and from 18.3 to 2.2 ng/ml after treatment with dexrazoxane and doxorubicin. Doxorubicinol was not detected in the sample obtained at 5 min postdosing, but metabolite levels rapidly increased to maximal levels within 10–30 min. Plasma doxorubicinol levels declined below the lower limit of quantitation (LLOQ, 3 ng/ml) at times (see Fig. 2); however, signal-to-noise ratios (s/n) equivalent to those found for doxorubicin (s/n, >8) at the LLOQ were observed at these concentrations. Therefore, the interday imprecision and accuracy of the assay for doxorubicinol would be expected to be similar to those for doxorubicin and would not exceed 20% at the lower values. Interestingly, plasma doxorubicinol levels declined to a minimum at approximately 8 h after treatment and then increased to a second maximum at 24 h (Fig. 2). A similar pattern has been observed in humans [19].

Table 1. Pharmacokinetic parameters of doxorubicin in dogs in the presence and absence of prior administration of dexrazoxane

Parameter	Saline	Dexrazoxane*
λ_z (h^{-1})	0.0234 ± 0.0029	0.0228 ± 0.0045
$t_{1/2}$, λ_z (h)	30.0 ± 4.0	31.2 ± 5.4
$AUC_{0-\infty}$ (ng h ml^{-1})	1,515 ± 332	1,465 ± 366
CL_s (ml min^{-1} kg^{-1})	17.2 ± 3.7	17.9 ± 4.6
CL_r (ml min^{-1} kg^{-1})	1.06 ± 0.56	1.66 ± 0.73
V_d, λ_z (l/kg)	44.4 ± 10.0	47.2 ± 8.0

Data represent mean values ± SD ($n = 4$)
* $P > 0.05$ (ANOVA)

Table 2. Plasma doxorubicinol/doxorubicin AUC_{0-t} ratios in dogs following the two treatments

	AUC_{0-t} (ng h ml^{-1})		
	M	P	M/P
Saline	464±242	1,440±311	0.307±0.095
Dexrazoxane	392±187	1,386±332	0.272±0.062*

M and P represent the metabolite (doxorubicinol) and the parent drug (doxorubicin), respectively
* $P > 0.05$ (ANOVA)

Table 3. Urinary excretion of doxorubicin and doxorubicinol by dogs following an i. v. dose of 1.5 mg/kg doxorubicin

	Percentage of dose (0–120 h)	
	Saline	Dexrazoxane
M	2.6 ±1.7	2.8 ±1.6
P	6.0 ±3.2	9.1 ±4.3
M+P	8.6 ±4.8	11.9 ±5.9
M/P ratio	0.416±0.097	0.255±0.069*

M and P represent the metabolite (doxorubicinol) and the parent drug (doxorubicin), respectively
* $P > 0.05$ (ANOVA)

For assessment of the effect of dexrazoxane on doxorubicin metabolism, the AUC_{0-t} value was computed for both the parent drug and the metabolite (Table 2). The metabolite AUC_{0-t} value was approximately 30% of that of the parent drug for both treatment arms and indicated significant biotransformation of doxorubicin to its 13-dihydro metabolite. Neither the doxorubicinol AUC_{0-t} value nor the AUC_m/AUC_p ratios were found to be significantly different ($P > 0.05$) between saline- and dexrazoxane-treated dogs.

Urinary excretion of doxorubicin and doxorubicinol

Table 3 provides the percentage of the delivered dose that was excreted in urine as the parent drug or metabolite and the M/P ratios for each treatment arm. High intersubject variability was observed in the percentage of the dose excreted (%CV, ~50) following either treatment sequence. The mean total urinary excretion of doxorubicin plus doxorubicinol was 8.6% and 11.9% of the delivered dose for the saline- and dexrazoxane-treated animals, respectively. Less variability was observed in the M/P ratios (%CV, <27%). The mean M/P ratios were 0.416 and 0.255 for the saline and dexrazoxane groups, respectively. Consistent with total urinary excretion, ANOVA revealed no significant difference ($P > 0.05$) in mean urinary M/P ratio of the two groups.

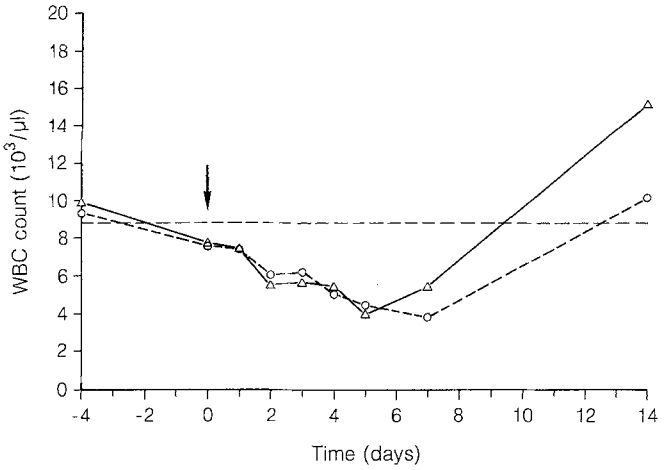

Fig. 3. Effect of doxorubicin on the mean WBC in dogs following its administration after saline or dexrazoxane ($n = 4$). The *horizontal line* represents the mean predose value and the *arrow* indicates the time of dosing. Normal values range between 6,000 and 17,000 WBC/μl. △, Saline/doxorubicin; ○, dexrazoxane/doxorubicin

Effect on WBC

The normal range reported for WBC in dogs is 6,000–17,000/μl whole blood, with the mean value being 11,500/μl [26]. A plot of the mean WBC as a function of time is provided in Fig. 3. The plot demonstrates that the nadir was reached between days 5 and 7, with values returning to basal (or higher than predose) levels by day 14. Unfortunately, measurements were inadvertently omitted on day 14 of the second period, which resulted in an n value of 2 for this sampling interval. Although no statistical evaluation was made, no obvious trend was apparent that suggested a difference between the two treatments.

Discussion

The present pharmacokinetics study was performed to assess the effects of prior administration of dexrazoxane on the metabolism, distribution, and excretion of doxorubicin. For clinical relevance, it was necessary that a species be chosen in which doxorubicin exhibits pharmacokinetic characteristics similar to those observed in man. The dog was selected as the model on the basis of previous work by Oosterbaan et al. [23], who have demonstrated that both the terminal half-life (24–30 h) and the volume of distribution (~65 l/kg) of doxorubicin in dogs appear to be similar to those reported for man [6, 12]. A metabolic similarity is also evident, since doxorubicinol, the principal metabolite in man, also occurs as a major systemically circulating species in dogs. Furthermore, the renal clearance and nonrenal clearance components for doxorubicin in dogs [23] reflect the ratio (~0.09) observed in man [6]. Thus, the dog appears to be an acceptable model to study this drug interaction.

The doses of doxorubicin and dexrazoxane were selected on the basis of the toxicity of doxorubicin in dogs and the clinical experience of Speyer et al. [27]. Doxorubicin

doses of ≥ 2.0 mg/kg are acceptable for short-term studies (<72 h) but are often lethal within 3 days of drug administration. A 1.5-mg/kg dose results in an acceptable level of toxicity and equates to the standard 60-mg/m^2 regimen for humans, assuming a surface area of 1.7 m^2 and a body weight of 70 kg. Dexrazoxane is injected no earlier than 30 min prior to doxorubicin administration and is given in multiples of the doxorubicin dose, the upper ratio being 20:1. Due to the variability inherent in doxorubicin pharmacokinetics [6, 19], small treatment effects may not be discernable; thus, selection of the 20:1 ratio was based on the premise that the highest ratio should produce the greatest effect.

A salient characteristic of doxorubicin is its propensity to bind to tissues. This property results in extensive exposure of tissue to the drug. The degree of exposure undoubtably has important ramifications in both the toxicity and therapeutic effects associated with this anthracycline. Using the $AUC_{0-\infty}$ value as a relative measure of drug exposure and V_d, λ_z values as a measure of tissue binding, we evaluated the effect of dexrazoxane on these two parameters. Neither the AUC value nor the V_d, λ_z values showed any significant ($P > 0.05$) treatment-related differences. The power to detect a 50% difference in AUC between the treatment means was 70%, which was apparently consistent with the a priori estimate obtained during the design of the study. This suggests that dexrazoxane does not influence drug exposure, tissue binding, or the general disposition characteristics of doxorubicin in dogs. More importantly, the CL_s value, which encompasses metabolic and renal processes, also remained unaltered in the presence of dexrazoxane and suggests no effect on the biotransformation of doxorubicin. This finding was further supported by the lack of significant treatment-related differences in either the AUC_m/AUC_p ratio or the renal excretion of doxorubicinol.

A direct assessment of the effect of dexrazoxane on tissue exposure to doxorubicin was made by monitoring WBCs, assuming that the magnitude of WBC suppression was a function of drug exposure. Although this assumption has not yet been documented for doxorubicin, it does appear to be valid for the closely related anthracycline epirubicin [20]. In concurrence with the lack of change in $AUC_{0-\infty}$, V_d, λ_z, and C_s values, no difference was apparent between the two treatments in the degree of WBC suppression, the time to nadir, or the time of return to the normal range.

The absence of significant differences in plasma pharmacokinetic parameters demonstrates that dexrazoxane has no effect on the disposition of doxorubicin. Apparently, either the trace metals involved in the generation of cytosolic reduced nicotinamide adenine dinucleotide phosphate (NADPH) are not affected or a sufficient pool of NADPH is available throughout the period during which dexrazoxane effectively chelates these metals. Competitive effects also come into play, since the intracellular concentrations of as well as the binding constants for various cations differ. Indeed, the binding constant of the hydrolysis product of dexrazoxane (most active chelator) [13, 18] for Fe(II) is 3–4 orders of magnitude greater than the binding constant for Mg(II) [9]. The presence of a relatively high concentration of Fe(II) in addition to its higher binding constant may preclude significant chelation of Mg(II), which is the cation facilitating the formation of NADPH.

In conclusion, the present results show that drug exposure was similar for the two treatment arms. Therefore, coadministration of dexrazoxane is unlikely to modify the safety and/or efficacy of doxorubicin.

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