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Pharmacokinetic-toxicodynamic Relationships of Adriamycin in Rat: Prediction of Butylated Hydroxyanisole-mediated Reduction in Anthracycline Cardiotoxicity

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Abstract

Adriamycin, an anthracycline antibiotic, has broad antitumour activity with cumulative dose-dependent cardiotoxicity as its major side effect. This study was designed to quantify and correlate the cardiotoxicity and pharmacokinetics of adriamycin in-vivo in rat. The influence of antioxidant (butylated hydroxyanisole, BHA) co-therapy on the cardiotoxicity and disposition of adriamycin was also studied.

Cardiotoxicity was estimated by serially measuring serum creatine kinase (CK) levels after intravenous administration of adriamycin (4, 10, 20, and 30 mg kg^{-1}) and adriamycin-BHA (10 and 30 mg kg^{-1} each). Peak serum CK concentrations (CK_{max}) and area under serum CK-time curves (AUC_{CK}) were used as cardiotoxicity indices. Pharmacokinetic studies were undertaken by sampling plasma and urine from distinct groups of rats treated with [^{14}C]adriamycin (at the above doses) and [^{14}C]adriamycin-BHA (10 mg kg^{-1} each). Co-administration of BHA resulted in a significant reduction in CK_{max} and also resulted in a significant reduction in the renal clearance of adriamycin which was fully compensated by an increase in its metabolic clearance. A linear increase in the area under the plasma adriamycin concentration-time curves (AUC) with increasing adriamycin doses suggested dose-independent disposition of the drug. The most significant pharmacokinetic-toxicodynamic relationships included: $CK_{max} = 2{\cdot}25 \times 10^2 \exp(4 \times 10^{-4} \text{ AUC})$, $r^2 = 0{\cdot}941$, $P < 0{\cdot}05$ and $AUC_{CK} = 1{\cdot}3 \times 10^4 \exp(2 \times 10^{-4} \text{ } AUC_{0\text{-}tmax})$, $r^2 = 0{\cdot}918$, $P < 0{\cdot}05$ where, $AUC_{0\text{-}tmax}$ is the area under the adriamycin concentration–time curve from time 0 to the time of the peak CK level.

The results strongly indicate that the drug-induced cardiotoxicity is initiated shortly after dosing when drug concentrations are high and accumulates with continued exposure. The predicted cardiotoxic indices, obtained from altered adriamycin pharmacokinetic parameters as a result of BHA co-therapy, compared favourably with the observed values.

Adriamycin is an anthracycline antibiotic with a wide spectrum of anticancer activity (Calabresi et al 1985). The most common toxic effects include alopecia, stomatitis, myelosuppression, gastro-enteritis, and cardiac toxicity (Powis & Hacker 1991). Cardiac toxicity is the major dose-dependent toxicity; it leads to congestive heart failure which is usually fatal. Incidence of adriamycin-induced cardiotoxicity becomes unacceptable when the total cumulative dose administered approaches 450 to 550 mg m^{-2} toxicity (Powis & Hacker 1991). It has been reported that administration of adriamycin as a 48-h or 96-h intravenous infusion reduces the cardiotoxicity with no apparent alteration in the therapeutic efficacy (Hortobagyi et al 1982). Similarly, a reduced incidence of cardiac failure was observed after weekly adriamycin dosing schedules (von Hoff et al 1979). Although these observations might indicate that adriamycin plasma concentrations might be important in anthracycline cardiotoxicity, a quantitative correlation between these drug disposition characteristics and its cardiotoxicity have not yet been explored.

Adriamycin cardiotoxicity is commonly detected by electrocardiography-left ventricular ejection fraction measurements (Mason et al 1978) or histopathological assessment after endomyocardial biopsy (Billingham & Bristow 1984). Although histopathological assessment is a sensitive test for functional evaluation of the cardiac muscle, it is invasive, expensive and requires the services of a skilled histopathologist (Billingham & Bristow 1984). Serial monitoring of intracellular enzymes in serum, such as creatine kinase and lactate dehydrogenase, have also been utilized as markers for adriamycin cardiotoxicity (Preus et al 1988; Sharkey et al 1991). More recently, ^{111}In-labeled antimyosin antibody has been used for quantitative assessment of the adriamycin cardiac effect (Carrio et al 1991; Hiroe et al 1992).

The heart contains low levels of free-radical scavenging enzymes such as catalase and glutathione peroxidase (Powis & Hacker 1991); it would, therefore, seem plausible that adriamycin-induced free radical damage is most severe in the cardiac tissue (Lown et al 1982). In apparent support of this hypothesis, several antioxidants, including vitamin E and *N*-acetylcysteine, have been shown to reduce adriamycin-induced cardiomyopathy (Mimnaugh et al 1979; Doroshow et al 1982). The effects of the common food antioxidant, butylated hydroxytoluene (BHA), on adriamycin cardiotoxicity and antitumour activity have not been evaluated, however. Because this antioxidant forms a part of the regular diet, it would be beneficial to examine its influence on anthracycline cardiotoxicity.

The goals of this study were to quantify and correlate the cardiotoxicity and pharmacokinetics of adriamycin in-vivo and to examine the effect of co-administration of antioxidant

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(BHA) on the cardiotoxicity of adriamycin. The extent of cardiotoxicity after administration of adriamycin to rats was measured by monitoring serial changes in serum creatine kinase (CK) with time. The toxicity indices obtained from serum CK measurements were correlated with various adriamycin pharmacokinetic parameters. These relationships were validated by predicting the cardiotoxicity of adriamycin-BHA using the pharmacokinetic parameters obtained from such co-administration and comparing the results with experimentally determined values. It has previously been shown that this antioxidant co-therapy does not inhibit the tumouricidal activity of adriamycin in an in-vitro cell survival assay (Vora et al 1996).

Materials and Methods

Chemicals

[14-^{14}C]Adriamycin was purchased from Amersham (Arlington, IL, USA). BHA, serum creatine kinase (CK) assay reagent (Kit #47-20) and L-glutamine were obtained from Sigma (St Louis, MO, USA). Adriamycin hydrochloride was generously provided by Adria Laboratories (Columbus, OH, USA) and hydroxypropyl-β-cyclodextrin by Pharmatec (Alachua, FL, USA). PE10 catheters, heparinized and non-heparinized capillary tubes, reagents and chemicals were purchased from Fisher Scientific (Pittsburgh, PA, USA).

Preparation of BHA-hydroxypropyl-β-cyclodextrin complex

The aqueous solubility of BHA was enhanced by formation of inclusion complex with hydroxypropyl-β-cyclodextrin, as described elsewhere (Vora & Boroujerdi 1995). This complex was used to co-administer BHA with adriamycin throughout this study.

Toxicodynamic studies

Serial changes in serum CK levels were evaluated in a cross-over design in Sprague–Dawley rats (150-200 g, Charles River Laboratories, Cambridge, MA, USA). Rats were anaesthetized with sodium pentobarbital (Anthony Products Co., Arcadia, CA, USA; 30 mg kg^{-1}, i.p.) and the lateral caudal vein cannulated with polyethylene tubing (PE10, 14 cm long) using a 19-gauge needle as a trocar. Approximately 1 h after cannulation (when serum CK concentrations were found to have subsided to baseline values) thirty rats were treated with saline on day 1 (control) and 5–6 days later with either adriamycin (4, 10, 20, 30 mg kg^{-1}; n = 5 each) or adriamycin-BHA (10 mg kg^{-1} each or 30 mg kg^{-1} each, n = 5 each).

Blood samples (60–80 μL) were collected in non-heparinized capillary tubes 0, 2, 4, 6, 8, 24, and 48 h after dosing. The total volume of blood withdrawn from each animal during the course of a treatment was always < 2 mL. The patency of the cannula was maintained between sampling by introduction of heparinized saline (100 U mL^{-1}). Before collection of each sample, the heparinized saline present in the cannula was discarded together with at least 3 drops of blood in order to minimize the contamination of the sample with the anticoagulant. Rats were maintained under anaesthesia by occasional administration of sodium pentobarbital (i.v.) for 8 h when the cannula was removed and the animals were left to recover. The 24-h and 48-h samples were drawn from the caudal vein of conscious rats using a 27 gauge needle attached to a tuberculin syringe. Blood samples were allowed to clot; serum was separated by centrifugation at 10 000 *g* for 4 min and stored at −20°C until analysis (usually within 2 weeks of collection). CK was assayed using commercially available reagents.

Pharmacokinetic studies

Plasma studies. Pharmacokinetic studies were performed in a separate group of male rats (150-200 g) using [14-^{14}C]adriamycin (spec. act. 0·2 μCi mg^{-1}). Radiolabelled adriamycin (4, 10, 20 or 30 mg kg^{-1}) or adriamycin-BHA (10 mg kg^{-1} each) in saline was administered by caudal vein injection to conscious rats (n = 5 per dose group for each of plasma and urine sampling).

Plasma sampling was accomplished by caudal vein cannulation in conscious rats as described above. Animals were dosed on the opposite caudal vein and blood samples (50-140 μL) were collected via heparinized capillary tubes at 5, 10, 15, 30, 45, 60, 90, 120 and 240 min in a 1·5 mL microcentrifuge tube to minimize adsorption of adriamycin. The cannula was then removed and samples were drawn from the caudal vein at 6, 8, and 10 h using a 27-gauge needle and tuberculin syringe rinsed with heparin (100 units mL^{-1}). The total volume of blood withdrawn from each animal was < 2 mL. A final blood sample was collected at 24 h by decapitation. Blood samples were centrifuged (10 000 *g*, 4 min); plasma was separated and stored at −20°C until analysis.

Urine analysis. For urine collection, a distinct group of rats was acclimatized to metabolic cages overnight before dosing. After dosing urine and cage washings were collected in 15-mL polypropylene tubes for the intervals: 0–1/2 h, 1/2–1 h, 1–2 h, 2–3 h, 3–4 h, 4–6 h, 6–8 h, 8–10 h, 10–24 h, 24–32 h and 32–48 h. The volume of each sample was noted and the samples were stored at −20°C until analysis.

Analysis of biological samples

Because the plasma samples collected were relatively small, sample clean-up was not attempted. The samples were assayed directly on an HPLC system consisting of a Waters (Milford, MA, USA) 501 solvent delivery system, Hitachi (Danbury, CT, USA) AS-2000 autosampler, Novapak C_{18} cartridge with a radial compression module (Model 8 × 10, Waters) and a guard column packed with μBondapak C_{18} (Waters). The mobile phase was 0·06 M monobasic potassium phosphate (pH 4.0)-acetonitrile (70:32.5, v/v) at a flow rate of 1 mL min^{-1}. The column eluent was fractionated every minute (1 mL) mixed with Scintiverse E (4 mL) and the radioactivity was measured with a liquid scintillation counter (Packard LSC 3501, Meriden, CT, USA) and corrected for background. No loss in counting efficiency was noted with this mixture of scintillator and mobile phase. To confirm the retention time of adriamycin, a radiolabelled standard solution was routinely injected after every 5th–7th sample injection, when adriamycin was consistently found to elute in the 5th fraction. The inter- and intra-day variability was found to be below 6%. The guard column packing material and filters were regularly changed after every 10 assays.

The total radioactivity in urine was determined by liquid scintillation counting (LSC). Urine samples (100 μL, in duplicate) were added to Scintiverse E scintillation cocktail (7 mL) and vortex mixed. Each sample was counted in a liquid

scintillation counter for 20 min and the counts corrected for background. The amount of adriamycin excreted unchanged in urine was determined by analysing 400 μL of each sample by HPLC. The chromatographic system and the mobile phase were similar to that described above, except that a Radiomatic A-140K radioactivity detector (Packard) was used on-line. The inter- and intra-day variability was found to be below 5%. The total amount of metabolites excreted in urine was obtained from the difference between the total radioactivity and the radioactivity present in the form of unchanged adriamycin.

Pharmacokinetic data analysis

The radioactivity (counts min^{-1}) present in plasma and urine was normalized to a body weight of 250 g. Plasma and urine counts obtained by HPLC and LSC were converted to μg mL^{-1} of adriamycin or adriamycin equivalents (i.e. adriamycin + labelled metabolites) using the specific activity of injected dose (0.2 μCi mg^{-1}).

Plasma adriamycin concentration–time data and cumulative amount of adriamycin excreted unchanged in urine-time data for each dose group were pooled and subjected to simultaneous curve fitting with biexponential equations (2-compartment open model with instantaneous input; Gibaldi & Perrier 1982):

$$C_p = \frac{Dose}{Vc}\sum_{l=1}^{n}\frac{(k_{21}-\lambda_l)}{\prod_{\substack{i=1\\ i\neq l}}^{n}(k_{21}-\lambda_l)}e^{(-\lambda_l t)} \quad (1)$$

$$Au = k_e\,Dose\frac{k_{21}}{\prod_{i=1}^{n}\lambda_i} + K_e\,Dose\sum_{l=1}^{n}\frac{(k_{21}-\lambda_l)}{\prod_{\substack{i=1\\ i\neq l}}^{n}-\lambda_l(\lambda i-\lambda_l)}e^{(-\lambda_l t)} \quad (2)$$

where C_p is the plasma adriamycin concentration (μg mL^{-1}) at time t (h), λ_i or λ_l are disposition rate constants (h^{-1}), k_{21} is the transfer rate constant between the peripheral and the central compartment (h^{-1}), k_e is the renal excretion rate constant (h^{-1}), Au is the cumulative amount (μg) of unchanged adriamycin excreted in urine at time t, and n is the number of exponents in the model (n = 2 for two-compartment model).

Parameter estimates were obtained using PCNonlin (Version 4.2, ClinTrials, Lexington, KY, USA). The resulting fit was examined by visually inspecting the curves and the data points, examining the Akaike's information criterion (AIC) values and the condition number, and evaluating the standard errors of the estimated parameters. Area under plasma adriamycin concentration-time curve was calculated by use of the trapezoidal rule from time 0 to 24 h (AUC) and from time 0 to the time of peak serum CK concentration (AUC_{0-tmax}). Similarly, area under the serum CK concentration-time profile was calculated by use of the trapezoidal rule from time 0 to 48 h (AUC_{CK}). Plasma clearance (CL, mL h^{-1}), overall elimination rate constant (k_{10}, h^{-1}), transfer rate constant between central and peripheral compartment (k_{12}, h^{-1}), renal clearance (CL_r, mL h^{-1}) and metabolic clearance (CL_m, mL h^{-1}) were calculated as shown below (Gibaldi & Perrier 1982):

$$CL = Dose/AUC \quad (3)$$

$$k_{10} = \lambda_1\lambda_2/k_{21} \quad (4)$$

$$k_{12} = \lambda_1 + \lambda_2 - k_{10} - k_{21} \quad (5)$$

$$CL_r = k_eCL/k_{10} \quad (6)$$

$$CL_m = CL - CL_r \quad (7)$$

Pharmacokinetic-toxicodynamic correlation

The anthracycline-induced cardiotoxicity measured by chronological changes in serum CK concentrations resulted in estimation of CK_{max}, the peak serum CK concentration (units L^{-1}), representing the instantaneous myocardial injury resulting from intravenous bolus administration of adriamycin, and AUC_{CK}, which represents the cumulative myocardial damage within 48 h of adriamycin dosing.

The pharmacokinetic parameters investigated included AUC (μg h mL^{-1}), AUC_{0-tmax} (μg h mL^{-1}), and initial plasma concentration, C_{max} (μg mL^{-1}). These parameters were correlated with the toxicity markers in an attempt to correlate the disposition with the dynamics of the drug.

Statistical analyses

Data are presented as mean ± s.d. of replicate experiments. Individual group comparisons were conducted using the two-tailed Student's *t*-test as appropriate. Multiple group comparisons were performed by one-way analysis of variance in conjunction with the Newman-Keuls test for post-hoc analysis.

Results

Toxicodynamic studies

Preliminary studies indicated that serum CK concentrations returned to their baseline values within 1 h of caudal vein cannulation. Sodium pentobarbital anaesthesia did not alter the baseline CK levels in rats (Fig. 1a). The peak serum CK concentrations (CK_{max}) increased as the dose of adriamycin administered increased, reaching a maximum between 2 and 8 h after drug administration (Fig. 1b). The overall myocardial damage resulting from anthracycline administration, as measured by area under serum CK-time curve (AUC_{CK}), increased with increasing doses of adriamycin.

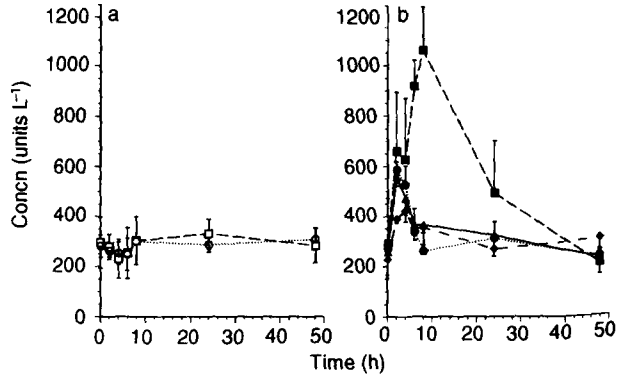

FIG. 1. Temporal profile of serum CK concentrations in saline- and adriamycin-treated rats. (a) There was no significant difference in baseline CK concentrations after administration of saline to pentobarbital anaesthetized (□, n = 12) and conscious rats (○, n = 4) (mean ± s.d.). (b) There was a dose-related increase in serum CK concentrations in rats treated with increasing doses of adriamycin. ◆ 4, ▲ 10, ● 20, ■ 30 mg kg^{-1}). n = 5.

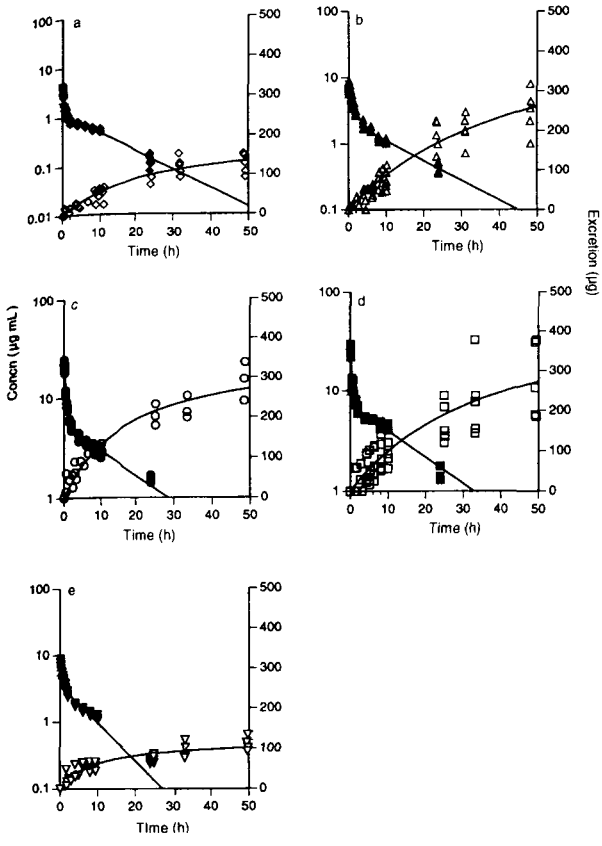

FIG. 2. Plots of plasma adriamycin concentration (C_p, closed symbols) and cumulative amount of unchanged adriamycin excreted in urine (A_u, open symbols) against time for various doses of anthracycline. a. 4, b. 10, c. 20, d. 30, e. 10 mg kg^{-1} each of adriamycin and BHA .

Administration of adriamycin-BHA resulted in a significant reduction in anthracycline cardiotoxicity. The use of 10 mg kg^{-1} concentrations of each of adriamycin and BHA resulted in a 29% reduction in maximum serum CK concentration (558 ± 36 to 450 ± 51, $P < 0.05$). Similarly, co-administration of BHA with adriamycin at 30 mg kg^{-1} each, resulted in a 41% decrease in peak CK concentrations (from 1062 ± 185 to 627 ± 13, $P < 0.05$).

Pharmacokinetic studies

The plasma concentration-time curves indicated an initial rapid decline in adriamycin concentration, followed by a more gradual reduction in plasma concentration, suggesting biexponential disposition of adriamycin. Correspondingly, the cumulative amount of adriamycin excreted unchanged in urine increased over time (Fig. 2). The plasma adriamycin concentration (C_p) and cumulative amount of adriamycin excreted (Au) vs time data were simultaneously fitted using equations 1 and 2. The resulting parameters are summarized in Table 1.

The volume of central compartment (Vc) remained relatively unaltered at the various doses, except for the smaller than expected value at 20 mg kg^{-1} adriamycin. Likewise, the plasma clearance of adriamycin (CL) remained essentially unaltered across the dose range evaluated, except for the group receiving adriamycin-BHA, which showed a significant increase. Indeed, a plot of AUC against dose of adriamycin administered alone was linear ($r^2 = 0.984$, $P < 0.05$). The renal clearance, however, decreased across the dose range as well as for the adriamycin-BHA treated group, possibly indicating renal toxicity of adriamycin (Powis & Hacker 1991). The cumulative amount of adriamycin excreted unchanged in urine increased slightly between 4 and 10 mg kg^{-1} adriamycin, but remained unaltered at 20 and 30 mg kg^{-1}. This parameter was also found to be significantly lower in the group treated with adriamycin-BHA. Metabolic processes appear, nevertheless, to compensate for this decline in renal performance. Thus, adriamycin disposition is likely to be dose-independent.

Pharmacokinetic-toxicodynamic relationships of adriamycin

The relationships between the intensity and time course of the drug-induced toxicity and the pharmacokinetic parameters of adriamycin resulting from combined plasma and urine analyses were investigated (Table 2, Fig. 3). The pharmacokinetic parameters after adriamycin-BHA administration were used to obtain values of cardiotoxic indices ('predicted' values). These values compared favourably with the experimentally observed values (Table 3).

Discussion

The magnitude of CK elevation in blood after cardiac injury has been shown to be directly proportional to the extent of damage in the musculature (Preus et al 1988; Sharkey et al 1991). Pentobarbital anaesthesia does not alter the serum levels of CK enzyme (Fig. 1a). Adriamycin, being highly water soluble, does not, furthermore, cross the blood-brain barrier and is, therefore, unlikely to influence total CK concentrations in serum.

Increasing doses of adriamycin caused an increase in serum CK concentrations in anaesthetized rats (Fig. 1b), with peak levels occurring between 2 to 8 h post-dose. There was a noticeable increase both in the peak CK concentrations in serum and in the total area under the CK-time curves, indicating increasing myocardial damage with increasing doses of adriamycin. The prolongation in the time to peak serum CK levels at higher doses of adriamycin might be indicative of the cumulative nature of the toxicity with continued enhancement of damage resulting from the circulating drug. We have already demonstrated that this antioxidant co-administration does not alter the tumouricidal activity of adriamycin in an in-vitro tumour cell survival assay (Vora et al 1996).

In order to establish a quantitative relationship between the cardiotoxicity and disposition characteristics, it was necessary to explore the pharmacokinetics of adriamycin and adriamycin-BHA. This provided an opportunity to study the linearity of anthracycline disposition which has not been attempted previously.

The plasma adriamycin concentration-time data and cumulative amount excreted unchanged with time data for each dose group were subjected to simultaneous curve fitting. The results show a decline in the volume of the central compartment at 20 mg kg^{-1} adriamycin. This observation is puzzling in the light of the relatively constant value of this parameter at other doses evaluated, including that for adriamycin-BHA (Table 1).

The plasma clearance of adriamycin-BHA (10 mg kg^{-1} each) showed a significant increase over that for adriamycin (10 mg kg^{-1}) alone. The renal clearance of adriamycin-BHA

Table 1. Pharmacokinetic parameters of adriamycin and adriamycin-BHA in rat after intravenous administration (mean ± (s.d.)).

Parameter	Adriamycin				Adriamycin-BHA
	4 mg kg^{-1}	10 mg kg^{-1}	20 mg kg^{-1}	30 mg kg^{-1}	(10 mg kg^{-1} each)
First disposition rate constant (h^{-1})	1·43 (1·26)	1·54 (0·49)	1·58 (0·16)	1·70 (0·18)	1·62 (0·51)
Second disposition rate constant (h^{-1})	0·033 (0·01)	0·018* (0·01)	0·014* (0·01)	0·039 (0·02)	0·067*,† (0·02)
Volume of the central compartment (mL)	265·14 (68·62)	276·17 (28·25)	187·40* (4·93)	239·52 (5·50)	280·59 (19·79)
Transfer rate constant between peripheral and central compartment (h^{-1})	0·50 (0·31)	0·46 (0·16)	0·12 (0·06)	0·15* (0·10)	0·50 (0·24)
Transfer rate constant between central and peripheral compartment (h^{-1})	0·87 (0·96)	1·04 (0·34)	1·30 (0·13)	1·14 (0·14)	0·97 (0·32)
Overall elimination rate constant (h^{-1})	0·094 (0·05)	0·059 (0·02)	0·179* (0·06)	0·438* (0·09)	0·217*,† (0·04)
Renal excretion rate constant (h^{-1})	0·015 (0·01)	0·009* (0·001)	0·019 (0·005)	0·014 (0·003)	0·008* (0·002)
Area under the plasma adriamycin concentration–time curves (μg h mL^{-1})	12·23 (0·58)	33·60 (1·97)	78·04 (2·88)	101·67 (1·37)	26·40† (2·17)
Plasma clearance (mL h^{-1})	81·75 (8·35)	74·40 (4·56)	64·07* (11·10)	73·77 (13·69)	94·70*,† (9·07)
Renal clearance (mL h^{-1})	13·44 (1·27)	12·49 (0·26)	6·84* (0·85)	2·34* (0·72)	3·87*,† (0·37)
Metabolic clearance (mL h^{-1})	68·32 (7·20)	61·92 (4·51)	57·23 (11·20)	71·43 (19·60)	90·83*,† (8·74)
Total amount of adriamycin + labelled metabolites in urine (μg)	155·70 (18·39)	392·79 (28·89)	699·64 (56·20)	914·86 (113·23)	324·25† (68·61)

*$P < 0.05$ compared with 4 mg kg^{-1} adriamycin. †$P < 0.05$ compared with 4 mg kg^{-1} adriamycin.

Table 2. Pharmacokinetic-toxicodynamic relationships of adriamycin in the rat.

Peak serum creatine kinase concentration	$= 2.26 \times 10^2 \exp(4.08 \times 10^{-4}\ AUC)\ r^2 = 0.941$*	(II-1)
Peak serum creatine kinase concentration	$= 2.21 \times 10^{-2}\ AUC_{0\text{-}tmax} + 433.12\ r^2 = 0.657$	(II-2)
Peak serum creatine kinase concentration	$= 3.99 \times 10^2 \exp(2.34 \times 10^{-2}\ C_{max})\ r^2 = 0.702$	(II-3)
Area under serum creatine kinase concentration–time curve	$= 1.31 \times 10^4 \exp(8.31 \times 10^{-5}\ AUC)\ r^2 = 0.501$	(II-4)
Area under serum creatine kinase concentration–time curve	$= 1.33 \times 10^4 \exp(1.99 \times 10^{-4}\ AUC_{0\text{-}tmax})\ r^2 = 0.918$*	(II-5)

*$P < 0.05$.

was, however, found to be significantly lower than that for adriamycin. This suggests an increase in metabolic processes. It is likely that BHA might be involved in the reduction of adriamycin renal clearance or in the increase of its metabolic transformation. This is not unusual because BHA has been shown to increase the biliary excretion of acetaminophen conjugates in pre-treated rats (McLaughlin & Boroujerdi 1987) and dietary BHA has been shown to increase the specific activity of cytochrome P450 in microsomal liver fraction (Benson et al 1978) and hepatic glutathione-*S*-transferase (Demkowicz-Dobrazanski et al 1984).

The renal clearance of adriamycin was found to decline significantly at higher doses of the anthracycline, most notably at 20 and 30 mg kg^{-1}. This might result from the known nephrotoxicity of adriamycin (Powis & Hacker 1991). The plasma clearance did not, however, decline significantly. It is likely that metabolic clearance might increase at higher doses, masking the reduction in renal performance. This increase might not be evident in the variability of the data (Table 1). AUC bore a linear relationship with dose administered ($r^2 = 0.984$), however, suggesting linear disposition of adriamycin in rat. These parameters were found to be comparable with those reported previously (Bapat & Boroujerdi 1993).

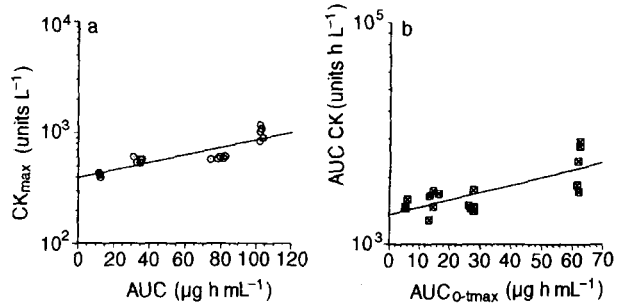

FIG. 3. Pharmacokinetic-toxicodynamic relationships of adriamycin. a. Plot showing the exponential relationship between peak serum CK concentration (CK_{max}) and area under the plasma adriamycin concentration-time curve (AUC). b. Plot of area under the serum CK concentration-time curves (AUC_{CK}) against area under plasma adriamycin concentration-time curve from time 0 to time of maximum CK concentration (AUC_{0-tmax}).

The pharmacokinetic parameters were correlated with the cardiotoxicity markers (Table 2, Fig. 3). The exponential nature of the reported relationships and the prolongation of the time required to reach maximum serum CK levels at higher doses of adriamycin indicate the increasing severity of cardiac

Table 3. Comparison of predicted and observed cardiotoxicity indices of adriamycin (using equations II-1 and II-5).

Cardiotoxicity index	Predicted (units L^{-1})	Observed (units L^{-1})	Difference (%)
Peak serum creatine kinase concentration	431	450	4
Area under serum creatine kinase concentration–time curve	$1{\cdot}34{\cdot}10^3$	$1{\cdot}50{\cdot}10^3$	11

injury with increasing doses. It is likely that the cardiotoxicity begins with the initial impact of the drug molecules on the heart musculature or at least within the first few hours post-dose.

The predictive capabilities of the two most significant relationships (Fig. 3) were evaluated by incorporating the altered pharmacokinetic parameters obtained from adriamycin-BHA administration and calculating the resulting toxicodynamic indices. These predicted toxicity markers were found to be in close agreement with the experimentally observed values (Table 3), demonstrating the utility and predictive capabilities of such relationships.

In conclusion, this study demonstrates the feasibility of establishing pharmacokinetic-toxicodynamic relationships of anthracyclines for possible prediction of drug-induced toxicity. Co-administration of the antioxidant resulted in a pronounced reduction in adriamycin cardiotoxicity without modulating its cytotoxic activity. The dose-independence of adriamycin disposition in the rat was also demonstrated.

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