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ORIGINAL ARTICLE

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Metabolism of dexrazoxane (ICRF-187) used as a rescue agent in cancer patients treated with high-dose etoposide

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Abstract *Purpose*: The study was undertaken to determine the metabolism of dexrazoxane (ICRF-187) to its one-ring open hydrolysis products and its two-rings opened metal-chelating product ADR-925 in cancer patients with brain metastases treated with high-dose etoposide. In this phase I/II trial dexrazoxane was used as a rescue agent to reduce the extracerebral toxicity of etoposide. *Methods*: Dexrazoxane and its one-ring open hydrolysis products were determined by HPLC and ADR-925 was determined by a fluorescence flow injection assay. *Results*: The two one-ring open hydrolysis intermediates of dexrazoxane appeared in the plasma at low levels upon completion of dexrazoxane infusion and then rapidly decreased with half-lives of 0.6 and 2.5 h. A plasma concentration of 10 μ*M* ADR-925 was also detected at the completion of the dexrazoxane i.v. infusion period, indicating that dexrazoxane was rapidly metabolized in vivo. A plateau level of 30 μ*M* ADR-925 was maintained for 4 h and then slowly decreased. The pharmacokinetics of dexrazoxane were found to be similar to other reported data in other settings and at lower doses. *Conclusions*: The rapid appearance of ADR-925 in plasma may make ADR-925 available to be taken up by heart tissue and bind free iron. These results suggest that the dexrazoxane intermediates are enzymatically metabolized to ADR-925 and provide a pharmacodynamic basis for the antioxidant cardioprotective activity of dexrazoxane.

Keywords Dexrazoxane · Metabolism · ADR-925 · Etoposide · Doxorubicin

Abbreviations $AUC_{0\rightarrow\infty}$ Area under the curve from time zero to infinity · C_{max} Concentration of drug at time zero · Cl_{tot} Total clearance · *DHOase* Dihydroorotase · *DHPase* Dihydropyrimidine amidohydrolase or dihydropyrimidinase · *HPLC* High-pressure liquid chromatography · K_f Equilibrium formation constant · P_{oct} Octanol-water partition coefficient · $t_{1/2}$ Half-time · t_r Retention time

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Introduction

Dexrazoxane (ICRF-187, Zinecard, Cardioxane; Fig. 1) is used clinically to reduce doxorubicin-induced cardiotoxicity [14, 20, 39, 40]. The clinical use of dexrazoxane arose as a result of a series of careful preclinical animal studies that have been described in recent reviews [20, 22]. Dexrazoxane likely acts as a cardioprotective agent by diffusing into the cell and hydrolyzing to its one-ring open intermediates **B** and **C**, and then to its fully rings-opened metal iron-chelating form ADR-925 (Fig. 1), which has a structure similar to that of EDTA. ADR-925 may act by chelating free iron or displacing iron bound to the iron-doxorubicin complex [1, 6, 14, 20], thus reducing iron-based oxygen radical formation. Considerable evidence has accumulated that the cardiotoxicity of doxorubicin is due to iron-based oxygen radical-induced oxidative stress on the heart muscle which is relatively low in antioxidant enzymes [5, 33, 34]. We and others have shown that the Fe^{3+}-doxorubicin complex is able to be reductively activated and redox cycle to produce the extremely damaging hydroxyl radical in a Fenton-type reaction [7, 9, 33, 34].

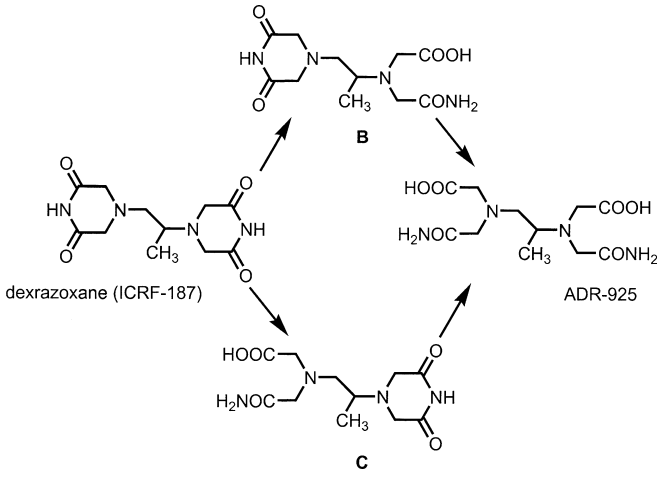

Fig. 1 Scheme for the metabolic hydrolysis of dexrazoxane (ICRF-187) to intermediates **B** and **C**, and its strongly metal ion-chelating form ADR-925

Our previous spectrophotometric and HPLC studies [11, 12] have shown that under physiological conditions dexrazoxane is only slowly hydrolyzed to **B** and **C** ($t_{1/2}$ 9.3 h at 37°C and pH 7.4) and the final hydrolysis product ADR-925 ($t_{1/2}$ 23 h), according to the kinetic scheme shown in Fig. 1. Given the slow rate at which hydrolysis-activation occurs in vitro it is unclear how sufficient amounts of ADR-925 could be present in heart tissue to chelate iron and prevent oxygen radical damage before dexrazoxane is eliminated ($t_{1/2\beta}$ 4.2 ± 2.9 h in humans) [25]. Our previous in vitro tissue homogenate, isolated hepatocyte, and purified enzyme studies and our in vivo rat studies have shown that dexrazoxane, but not **B** or **C**, are metabolized by DHPase (EC 3.5.2.2) that is present in the liver and kidney, but not in the heart [10, 13, 15, 16, 17]. However, **B** and **C**, but not dexrazoxane, are both enzymatically hydrolyzed by purified DHOase (EC 3.5.2.3) [37] which is present in the heart, liver, kidney and blood.

Dexrazoxane is also a strong catalytic inhibitor of DNA topoisomerase II [18]. In preclinical studies dexrazoxane has been shown to antagonize etoposide and daunorubicin DNA strand breaks and cytotoxicity [30, 38] and doxorubicin cytotoxicity [19]. Our previous in vivo experiments have shown that dexrazoxane protects against etoposide-induced lethality and allows a 3.6-fold dose escalation of etoposide [26, 27]. Furthermore, in mice inoculated with tumor cells into the central nervous system, treatment with the combination of high-dose etoposide and dexrazoxane resulted in highly significant increases in life span [27]. Dexrazoxane is hydrophilic ($\log P_{oct}$ −1.8 [18]) and likely does not cross the blood-brain barrier, but lipophilic etoposide does. Thus, dexrazoxane protects normal tissues and is not able to antagonize etoposide in the central nervous system. The concept of dexrazoxane rescue from high-dose etoposide treatment was the subject of a small phase I/II clinical trial of patients with brain metastases from primary small-cell lung cancer that provided the patient population for this study.

The approval of dexrazoxane by the American Food and Drug Administration in May 1995 required a follow-up phase IV commitment to characterize the pharmacokinetics of the metabolites. To our knowledge there has been no published report concerning the pharmacokinetics of the metabolites other than our report on the metabolism and pharmacokinetics in the rat [15, 36]. It was not the object of this study to characterize the pharmacokinetics of dexrazoxane as this has been well studied in patients with [25, 29, 35] and without [4, 41] anthracycline treatment. The topic has also been recently reviewed [24]. Thus, plasma samples from patients undergoing dexrazoxane rescue from high-dose etoposide treatment were analyzed for its three hydrolyzed metabolites and for dexrazoxane (Fig. 1) in order to obtain a greater understanding of the metabolism and the mechanism of the protective effects of dexrazoxane.

Patients and methods

Patient eligibility and characteristics

The trial inclusion criteria were: histologically verified small-cell lung cancer together with CT- or MR-evaluable brain metastasis and age >18 years. In addition, only one prior chemotherapy regimen which included etoposide and which was terminated no later than 3 weeks prior to inclusion was permitted. The ECOG performance score had to be ≤ 2, and life expectancy >3 months. Adequate organ function to enter the trial was defined as: WBC $>3.0\times10^9$/l, platelets $>100\times10^9$/l, total bilirubin less than 1.25 times the upper normal limit, aspartate aminotransferase less than twice the upper normal limit, or less than five times the upper normal limit in the presence of verified liver metastases and serum creatinine less than 1.5 times the upper normal limit. The patient had to understand the objective of the study, and must have signed an informed consent. The study was approved by the Danish Medical Authorities and by the local ethics committee.

Three males and two females were included in this pharmacokinetic study. They had a median age of 67 years (range 58–74 years). They had a median weight of 68 kg (range 53–80 kg). Four patients had received one prior chemotherapy regimen consisting of etoposide, carboplatin and vincristine, while the fifth patient had been treated with one series of topotecan, carboplatin, cisplatin, etoposide and vincristine.

Therapy

Patients in the study group were treated every 3 weeks. The pharmacokinetic studies were done only on the first day of treatment. Three patients were dosed with 500 mg/m^2 etoposide and 1500 mg/m^2 dexrazoxane. One patient was dosed with 1000 mg/m^2 etoposide and 1000 mg/m^2 dexrazoxane, and one patient was dosed with 650 mg/m^2 etoposide and 1500 mg/m^2 dexrazoxane.

Both etoposide and dexrazoxane were administered through a vascular port catheter. Dexrazoxane was administered over 15 min, followed by the infusion of etoposide over 90 min. The etoposide was infused undiluted within 15 min of the completion of the infusion of dexrazoxane. All patients were treated i.v. 30 min prior to the infusion of dexrazoxane and etoposide with 80 mg Solu-Medrol (methylprednisolone sodium succinate; Pharmacia & Upjohn, Kalamazoo, Mich.) and 2 mg Tavegyl (clemastine; Novartis,

Basel, Switzerland), for prevention of anaphylactic reactions to the infusion of etoposide, and 100 mg Nizax (nizatidine; Eli Lilly, Indianapolis, Ind.) for protection of the gastric mucosa.

Materials

"Dexrazoxane hydrochloride (Cardioxane) for the clinical studies was obtained from Chiron (Amsterdam, The Netherlands) as a lyophilized powder in 500-mg vials. It was reconstituted in 25 ml sterile water, and the total amount of drug was then mixed with isotonic glucose to a total volume of 250 ml prior to infusion. Etoposide (Vepesid; Bristol-Myers Squibb, New York, N.Y.) was obtained as a ready-to-administer liquid solution (100 mg etoposide in 5 ml). Dexrazoxane hydrochloride and ADR-925 used in the HPLC assays were gifts from Adria Laboratories (Columbus, Ohio). Calcein ("high purity") was from Molecular Probes (Eugene, Ore.). HPLC-grade methanol and cobalt chloride hexahydrate were from Fisher (Nepean, Canada), and the 1-heptanesulfonic acid and 1-octanesulfonic acid sodium salts were from Sigma (St. Louis, Mo.).

HPLC separation of dexrazoxane, its one-ring open intermediates **B** and **C**, and ADR-925

The HPLC analysis of dexrazoxane and its one-ring open intermediates using a reversed-phase C_{18} column has been described in our previous studies [10, 11, 12, 15, 17, 36]. In order to compare the amount of ADR-925 obtained by HPLC and by the flow injection method, ADR-925 was determined ($n=4$) in the plasma of one patient by HPLC (t_r 6.2 min) under isocratic conditions (500 μ*M* Na_2EDTA/10 m*M* octanesulfonic acid, pH 2.8, 1 ml/min).

The HPLC calibration plots using integrated peak areas (10 to 300 μ*M* dexrazoxane, 2 to 60 μ*M* **B**, 2 to 30 μ*M* **C**, and 10 to 70 μ*M* ADR-925) were prepared by adding standards containing known amounts of dexrazoxane, **B**, **C** and ADR-925 (prepared as previously described [11]) to blank plasma. The calibration plots ($n=6$ or 7) for dexrazoxane, **B**, and **C** were linear (r^2 0.999, 0.996 and 0.993, respectively). Absolute recoveries from spiked plasma ranged from 90% to 100% over a 10 to 200 μ*M* dexrazoxane range. Similarly, the recovery of **B** and **C** ranged from 77% to 98%, and 81% to 100%, respectively, over the range of 5–50 μ*M*. The limits of quantitation of dexrazoxane, **B**, and **C** in plasma were 1, 2, and 2 μ*M* (estimated from three times the limit of detection), respectively. Errors shown are the SEM. Between-day variation in the slopes of the calibration plots was 11% (±SD) for dexrazoxane, and 2 and 0.3% (± average deviation) for **B**, and **C**, respectively. Accuracy, which depended upon the concentration of the analyte, was generally better than 4% [36].

Fluorescence flow injection analysis of ADR-925

The low molar absorptivity of ADR-925 [8] prevented its routine determination in plasma by HPLC. ADR-925 plasma levels were determined with a highly sensitive flow injection method [36] that used the fluorescent (λ_{ex} 496 nm, λ_{em} 517 nm) metal iron-chelating indicator calcein. Calibration plots of integrated peak areas ($n=6$) in the range 0 to 1.0 μ*M* ADR-925 were repeated every 3 h during analysis and were found to be linear ($r^2 > 0.997$). Absolute recoveries from spiked plasma ranged from 90% to 98% over a 5 to 50 μ*M* ADR-925 range. Between-day variation in the calibration plots was 2% (±SD). The limit of detection of ADR-925 in plasma was estimated to be 1 μ*M*.

Because the calcein assay measures only free ADR-925, experiments were also done to determine the relative amount of free ADR-925 in plasma. In preliminary experiments, it was shown that stirring the chelating resin Chelex (Sigma) with preformed Fe^{3+}-ADR-925 at pH 5.5 resulted in complete displacement of Fe^{3+} from its complex with ADR-925 within 20 min. Thus, the ADR-925 levels reported after Chelex treatment are total ADR-925 levels, and those before Chelex treatment are free ADR-925 levels.

Sample collection and treatment

Blood samples were drawn at predetermined times (0, 0.25, 0.50, 1.0, 2, 3, 4, 8, 12, 16 and 24 h) starting directly after completion of the infusion of dexrazoxane (defined as time zero). A blood sample was also taken as an HPLC control before the infusion of any drugs. Blood (5 ml) was collected in Vacutainers (BD, Franklin Lakes, N.J.) containing lithium heparin (143 IU). Blood sampling was from a peripheral vein during infusion of drugs, and after completion of treatment the blood was withdrawn from a vascular port catheter (Port-a-Cath). The plasma was separated by centrifugation at 650 *g* for 5 min and then split into two. The portions for analysis of dexrazoxane and its metabolites were generally treated with 10 μl 5 *M* HCl/ml plasma in order to bring the pH to 3 to reduce hydrolysis of dexrazoxane and its metabolites [11]. The treated samples were stored at −80°C. To precipitate plasma proteins, acetonitrile (Fisher, Markham, Canada) was added at a ratio of 2:1 (v/v). The samples were mixed, allowed to settle for 5 min and centrifuged at 8000 *g* for 10 min. The supernatant was removed, acidified to pH 3 with HCl, and evaporated to dryness under nitrogen. The samples for the ADR-925 analysis were reconstituted in water to their original volume just before analysis. Samples for dexrazoxane, **B**, and **C** analysis were reconstituted in 10 m*M* HCl/500 μ*M* Na_2EDTA to their original volume just prior to analysis. The dexrazoxane hydrochloride that was infused was found to contain less than 0.5% **B**, 0.2% **C**, and 0.3% ADR-925 (mol%). The levels of these hydrolysis products were so low that the levels detected in vivo could not have come from the formulated drug itself.

Data analysis

The biphasic plasma decay curves for dexrazoxane were fitted to a bolus i.v. two-compartment model (WinNonlin 1.1, Pharsight, Mountain View, Calif.) as previously described for dexrazoxane [4, 29]. The apparent elimination half-lives for intermediates **B** and **C** were determined by fitting the data to a bolus i.v. one-compartment single-exponential decay model. Errors quoted are SDs unless otherwise indicated.

Results

Dexrazoxane pharmacokinetics

Dexrazoxane displayed biphasic elimination kinetics (Fig. 2) as has been well described before [4, 25, 29, 41]. The results of dexrazoxane pharmacokinetic modeling for the five patients are given in Table 1. All patients but one were dosed at 1500 mg/m^2 of dexrazoxane. The data for the one patient who was dosed at 1000 mg/m^2 of dexrazoxane were included in Table 1 after multiplying the concentration of dexrazoxane and its metabolites by 1.5, as previous studies have shown that the elimination pharmacokinetic parameters are not influenced by dose [25, 29, 41]. The average plasma levels of dexrazoxane, **B**, **C** and ADR-925 obtained after dexrazoxane infusion (1500 mg/m^2) are plotted in Fig. 3 in order to show the appearance and disappearance of **B**, **C** and ADR-925 together on the same plot.

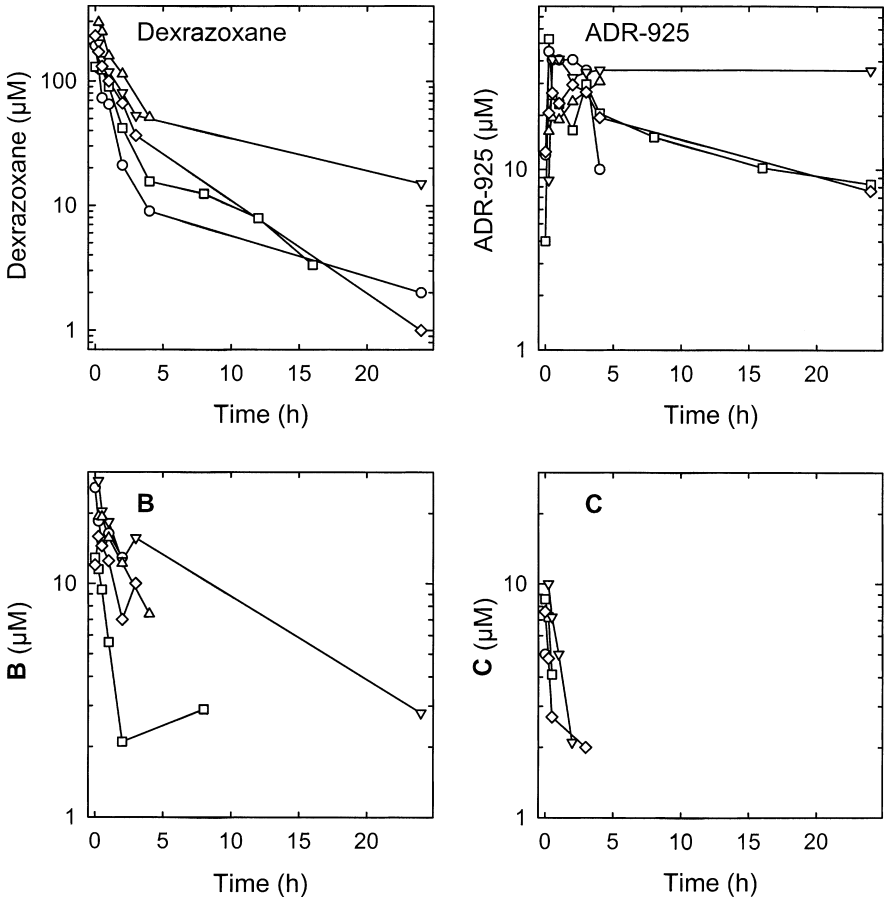

Fig. 2 Individual plasma concentrations of dexrazoxane, ADR-925, **B** and **C**. The data for one patient dosed at 1000 mg/m^2 were normalized to a dose of 1500 mg/m^2. Note that while the **B** and **C** plots are plotted on the same vertical scale, the dexrazoxane and ADR-925 plots are on different scales. (△ patient 1, ▽ patient 2, ♦ patient 3, □ patient 4, ○ patient 5)

Formation of the dexrazoxane metabolites

The intermediates **B** and **C** appeared in the plasma very quickly after dexrazoxane administration and then continuously decreased to below detectable limits (1 μ*M*) by 8 h (except for patient number 2 who also had a long $t_{1/2\beta}$ for dexrazoxane itself) and 3 h, respectively (Fig. 2). The level of **C** was a nearly constant 3–5% of dexrazoxane plasma levels. The level of **B** increased from an initial 8% to maximally 29% of the dexrazoxane plasma levels at 3 h. The semilog plots of the **B** and **C** data were approximately linear and thus the data were empirically fitted to a bolus i.v. one-compartment exponential decay model (Table 1). The average apparent half-lives for the disappearance of intermediates **B** and **C** had values of 2.5 ± 1.1 and 0.6 ± 0.2 h, respectively. The average apparent C_{max} values for intermediates **B** and **C** were 19.3 ± 5.2 and 9.1 ± 3.0 μ*M*, respectively. These are

Table 1 Dexrazoxane pharmacokinetics and the apparent half-lives of metabolites **B** and **C** of patients also treated with high-dose etoposide (*n* number of plasma concentration–time points for each patient that were used to obtain the pharmacokinetic parameters, *ND* not determined)

Patient	Dexrazoxane (mg/m^2)	Etoposide (mg/m^2)	Sex	Dexrazoxane						Metabolite **B**			Metabolite **C**		
				n	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	C_{max} (μ*M*)	$AUC_{0\rightarrow\infty}$ (μ*M*·h)	Cl_{tot} (ml·min^{-1}·m^{-2})	*n*	$t_{1/2}$ (h)	C_{max} (μ*M*)	*n*	$t_{1/2}$ (h)	C_{max} (μ*M*)
1	1500	500	M	5	1.3	ND[a]	314	580[d]	161	5	2.6	21.1	–	ND[b]	ND[b]
2	1500	500	M	6	0.9	16.4	215	1210	77	6	2.9	25.4	4	0.8	12.2
3	1500	650	F	7	0.7	4.3	210	460	202	6	3.9	14.4	4	0.5	6.3
4	1000	1000	M	8	0.8	5.9	152[c]	350[c]	266[c]	5	0.9	13.3[c]	3	0.5	8.9[c]
5	1500	500	F	7	0.5	9.8	165	270	345	5	2.1	22.1	–	ND[b]	ND[b]
Mean					0.8	9.1	211	570	210		2.5	19.3		0.6	9.1
SD					0.3	5.4	64	430	101		1.1	5.2		0.2	3.0

[a]For patient 1, the dexrazoxane beta phase was not determined as dexrazoxane plasma concentrations were only determined out to 4 h
[b]Metabolites **B** and **C** were below the limit of quantitation
[c]Normalized to a dose of 1500 mg/m^2 of dexrazoxane assuming that the values were proportional to the dose
[d]Not used in the calculation of the mean because beta phase pharmacokinetics could not be determined

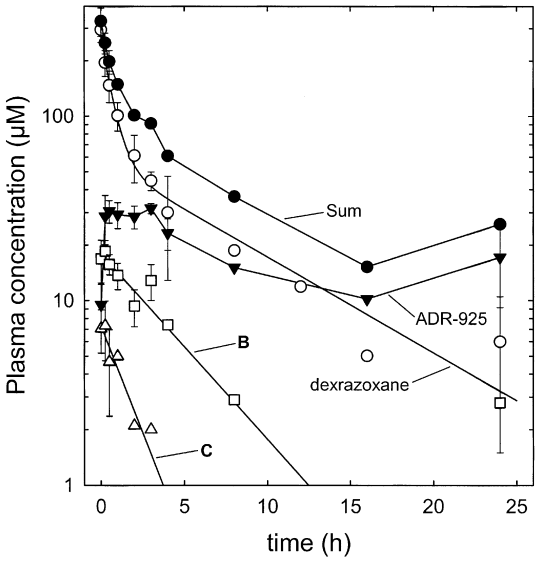

Fig. 3 Average plasma concentrations of dexrazoxane (○), **B** (□), **C** (△) and total ADR-925 (), and the sum of the concentrations of dexrazoxane, **B**, **C** and ADR-925 (Sum, ●) after dosing patients i.v. with 1500 mg/m^2 dexrazoxane. The smooth solid line was calculated from a two-compartment analysis of all the averaged data from all five patients. The best fit to this averaged data yielded $t_{1/2\alpha}$ 0.4 ± 0.1 h, $t_{1/2\beta}$ 5.8 ± 1.0 h, C_{max} 280 ± 16 μ*M*, $AUC_{0\rightarrow\infty}$ of 617 ± 48 μ*M*·h, and Cl_{tot} 151 ± 12 ml·min^{-1}·m^{-2}, where the errors are SEs obtained from the modeling. Error bars on the data points represent the SEs and where no error bars are shown the results are from a single patient (patient no. 4). The data at time zero are an average of six determinations from three patients, one of whom (patient no. 5) underwent four courses of treatment at 3-week intervals. For the last three courses of treatment for this patient, blood samples were only obtained at time zero. Other data points are averages from two to five data points on each of five patients. Least squares calculated straight lines are drawn through the averaged **B** and **C** data

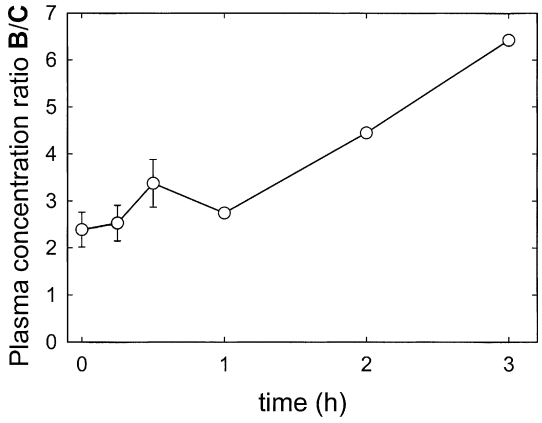

Fig. 4 Ratio of the average concentrations of plasma metabolite **B** to that of plasma metabolite **C** calculated from the data in Fig. 3

apparent values only because **B** and **C** are intermediates that are being formed, distributed, and eliminated simultaneously. It can also be seen from the data in Fig. 3 that the average concentration of **B** was always higher than that of **C**. The ratio **B**/**C** (Fig. 4) increased from an initial value of 2.4 to 6.4 at 3 h.

The average total ADR-925 levels are also plotted in Fig. 3. ADR-925 was already detectable (9.5 ± 2.8 μ*M*) in the plasma at the end of the dexrazoxane infusion period when the first sample was taken at t = 0 (Fig. 3). ADR-925 then rapidly increased nearly threefold to 29 ± 9 μ*M* at 15 min after the infusion, remained relatively constant for the next 4 h and then slowly deceased by about half at 24 h. By 16 h total ADR-925 levels exceeded that of dexrazoxane, **B** or **C**. Patient number 2 also showed much higher levels of ADR-925 at 24 h compared to the other two patients.

The ADR-925 levels shown in Figs. 2 and 3 are the total ADR-925 levels obtained after Chelex treatment of the plasma samples. The percentages of free and heavy metal-bound ADR-925 were determined by measuring ADR-925 levels before and after Chelex treatment of plasma samples collected at ten time-points for two patients. The values found ranged from 23% to 83% free ADR-925 (average 56 ± 6%) and indicated that a significant amount of the total ADR-925 present was bound to heavy metal ions. We showed that ADR-925-containing solutions picked up significant quantities of heavy metals when they were passed even briefly through a short stainless-steel needle. Thus, our data do not allow us to determine whether the source of this heavy metal bound to ADR-925 was from the blood sampling, which was carried out using stainless-steel needles, or whether it was from endogenous iron scavenged from the plasma.

While the calcein assay was not sensitive to dexrazoxane, **B**, or **C**, the possibility exists that other dexrazoxane-derived chelating metabolites that the calcein assay might have detected were formed. In order to test this possibility, an experiment was carried out on a single plasma sample obtained at 0.5 h after dexrazoxane infusion, which when concentrated contained sufficient ADR-925 to measure both by HPLC and by the calcein assay. The plasma concentration of ADR-925 was found to be 34 and 40 μ*M* using the HPLC and calcein assay, respectively. The agreement of these two values indicates that there were no other major detectable dexrazoxane-derived chelating metabolites formed in vivo.

Discussion

Pharmacokinetics of dexrazoxane

The average values (Table 1) of $t_{1/2\alpha}$ of 0.8 ± 0.3 h and a $t_{1/2\beta}$ of 9.1 ± 5.4 h determined in this study can be compared to $t_{1/2\alpha}$ values ranging from 0.17 to 0.47 h and $t_{1/2\beta}$ values ranging from 1.1 to 13.9 h determined with [25, 29, 35] and without [4, 41] anthracycline treatment. The $AUC_{0\rightarrow\infty}$ of 570 ± 430 μ*M*·h of this study can be compared to values of 500, 470, and 490 μ*M*·h at dexrazoxane doses of 900,1000, and 1580 mg/m^2 [4, 25, 35]. Similarly, Cl_{tot} was similar to values ranging from 111 to

265 ml·min^{-1}·m^{-2} [4, 25, 29]. This study was not specifically designed to test whether high-dose etoposide treatment affected dexrazoxane pharmacokinetics. However, the pharmacokinetics of dexrazoxane were similar to other reported data in other settings and at lower doses.

Metabolism of dexrazoxane to intermediates **B** and **C**

No other study in humans has shown the presence of the metabolites **B** and **C** after dexrazoxane treatment, although we have previously shown that **B** and **C** rapidly appear in the plasma of dexrazoxane-treated rats (maximally reaching 24% and 9%, respectively, of the dexrazoxane concentration at 2 h) [15, 36]. The initial rapid decline in plasma concentrations of **B** and **C** (Fig. 2) in humans paralleled that seen in our rat study [36] but was slower. The results of this study showing that the proportion of **B** ranged from an initial value of 8% to 29% of the dexrazoxane value at 4 h, and that the proportion of **C** was a nearly constant 3% to 5% of dexrazoxane levels, are not too different from those found in rats, suggesting that the two species have common dexrazoxane metabolic pathways. The **B**/**C** ratio of 2.4 (Fig. 4) seen at time zero and its increase with time to 6.4 at 3 h is also similar to that seen in our rat model in which an initial value of 2.7 at 5 min and 6.5 at 3 h was seen [15]. Again these results suggest that the metabolism in these two species is similar. We have previously shown that in dexrazoxane-treated isolated primary rat hepatocytes the initial **B**/**C** ratio is 2.7 [17]. We have also previously shown that purified DHPase catalyzes the ring opening of dexrazoxane to give **B**/**C** ratios in the range 2.9–6.1 depending upon the pH [10]. Thus the initial **B**/**C** ratio of 2.4 seen in this study is consistent with dexrazoxane being metabolized by DHPase as we have previously concluded in the in vivo rat model and rat hepatocyte model studies [15, 17].

Dexrazoxane undergoes a slow base-catalyzed hydrolysis (Fig. 1) to **B** and **C** ($t_{1/2}$ 9.3 h) and then to ADR-925 ($t_{1/2}$ 23 h) under physiological conditions (37°C and pH 7.4) [11, 12]. Given the slow rate of the in vitro hydrolysis of dexrazoxane under physiological conditions, little of the **B** and **C**, or ADR-925 seen in vivo could have been formed from base-catalyzed hydrolysis of dexrazoxane [11, 12]. Therefore, the appearance of these metabolites must have resulted from rapid metabolism of dexrazoxane, and intermediates **B** and **C**, respectively.

Metabolism of **B** and **C** to ADR-925

The presence of ADR-925, the presumably active metabolite of dexrazoxane, in the plasma of patients given dexrazoxane has not been previously determined. The data shown in Figs. 2 and 3 indicate that ADR-925 was rapidly produced after dexrazoxane administration, which suggests that either **B** or **C**, or both, were rapidly metabolized to ADR-925. The ADR-925 concentration-time plot (Fig. 3) also paralleled that of our rat study [36], except that peak levels were reached more slowly. The relative lack of change in the level of ADR-925 after 10 h suggests that ADR-925 was in a dynamic steady state. Recent results in our rat model [36] have also shown that ADR-925 is detected directly after the infusion period and likewise reaches a steady-state level. We have recently shown that DHOase, which is present in the heart, liver, kidney, and blood enzymatically hydrolyzes **B** and **C** (but not dexrazoxane) to ADR-925 [36]. Thus, DHPase and DHOase acting in succession on dexrazoxane to ultimately form ADR-925 (Fig. 1) may provide a mechanism by which dexrazoxane is activated.

Previous animal studies have shown that the timing of dexrazoxane dosing is critical for it to exert its protective effects [21, 23]. Maximal protective effects are seen when dexrazoxane is given between 3 h before and 3 h after daunorubicin treatment in Syrian golden hamsters [23]. Likewise, doxorubicin-induced cardiomyopathy is reduced in beagle dogs receiving dexrazoxane simultaneously compared to those receiving dexrazoxane 2 h after doxorubicin treatment [21]. These results were the basis for the recommendation that in a clinical setting the i.v. injection of doxorubicin should be given after completing the infusion of dexrazoxane and prior to a total elapsed time of 30 min (from the beginning of the dexrazoxane infusion). The results of this study showing that ADR-925 rapidly appeared in the plasma after dexrazoxane administration now provide a pharmacodynamic basis for this dosing schedule.

This study did not directly address how dexrazoxane exerts its doxorubicin cardioprotective effects in the heart. We have previously shown that while a suspension of rat primary hepatocytes [17] and supernatants of liver and kidney homogenates do enzymatically hydrolyze dexrazoxane to **B** and **C**, the heart does not [16]. DHPase has been shown to be responsible for this enzymatic hydrolysis [10, 13, 16, 17]. Dexrazoxane is cell-permeable [2] and should thus be readily taken up by tissues. The fact that ADR-925 rapidly appeared in the plasma (Fig. 3) indicates that dexrazoxane was rapidly metabolized in tissues and that these charged metabolites were sufficiently cell-permeable to be rapidly released into the blood. Thus, it follows that, even if dexrazoxane is not metabolized in the heart, the metabolites are probably sufficiently cell-permeable to be taken up by heart tissue and to be present at sufficiently high concentrations to chelate free iron in the heart and prevent the formation of the Fe^{3+}-doxorubicin complex or remove Fe^{3+} from the Fe^{3+}-doxorubicin complex. ADR-925, the rings-opened hydrolysis product of dexrazoxane and an analog of EDTA, is a strong chelator of Fe^{2+} (K_f $10^{10.0}$ M^{-1}) [28] and of Fe^{3+} (K_f $10^{18.2}$ M^{-1}) [3]. We have previously shown that ADR-925 is able to quickly ($t_{1/2}$ 1.7 min) and efficiently remove Fe^{3+} from its complex with doxorubicin [1, 6, 14, 20], and thus prevent iron-based

doxorubicin-mediated free radical oxidative stress on the heart muscle [5, 33, 34].

This study also did not directly address how dexrazoxane exerts its extracerebral etoposide-rescue effects. There is considerable in vivo evidence that doxorubicin-induced cardiotoxicity is due to iron-based oxidative stress [5, 7, 9, 33, 34]. Likewise, there is also some evidence that etoposide toxicity may also be due to oxidative stress, as it has been shown that etoposide can be activated by peroxidase [31, 32] and tyrosinase [42, 43] to form etoposide phenoxy free radicals. These radicals have been shown to deplete antioxidant cellular sulfhydryl compounds, the oxidation of which can lead to superoxide and H_2O_2 formation, leading to iron-based oxygen radical damage. Alternatively, the etoposide-rescue effect of dexrazoxane may be due to the fact that it is a strong catalytic inhibitor of topoisomerase II [18] which is able to antagonize [19, 30, 38] etoposide-induced cytotoxicity and cellular damage.

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