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ORIGINAL ARTICLE

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The use of serum levels of cardiac troponin T to compare the protective activity of dexrazoxane against doxorubicin- and mitoxantrone-induced cardiotoxicity

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Abstract *Purpose*: To compare the protective effect of dexrazoxane (DRZ) against cardiotoxicity induced by doxorubicin (DXR) and mitoxantrone (MTX). *Methods*: Adult male spontaneously hypertensive rats (SHR) were treated with 1 mg/kg DXR (i.v.) or 0.5 mg/kg MTX (i.v.), either alone or 30 min after 25 mg/kg DRZ (i.p.) weekly for up to 12 weeks. Animals treated with DXR alone either died ($n = 2$) or were killed ($n = 3$) at a cumulative dose of 10 mg/kg. The severity of cardiac lesions (cytoplasmic vacuolization and myofibrillar loss) were graded semiquantitatively by light microscopy on a scale of 0 to 3. *Results*: Cardiac lesions were observed in all SHR given DXR or MTX alone, and were attenuated in those given DRZ prior to either DXR (mean lesion scores 2.7 vs 1.5; $P < 0.05$) or MTX (mean lesion scores 2.0 vs 1.25; $P < 0.05$). Cardioprotection was also demonstrated by monitoring serum levels of cardiac troponin T (cTnT), which were elevated in all animals receiving DXR or MTX alone. These elevations were attenuated in SHR given the combination of DXR and DRZ (mean values 0.79 ng/ml vs 0.24 ng/ml; $P < 0.05$) and MTX and DRZ (mean values 0.19 ng/ml vs 0.04 ng/ml; $P < 0.05$). Biochemical studies have shown that both DXR and MTX form potentially cardiotoxic complexes with iron. ADR-925 (the hydrolysis product of DRZ) and other chelators (EDTA, diethylenetriaminepentaacetic acid and desferrioxamine) removed Fe(III) from its complex with MTX or DXR. *Conclusions*: The present study showed that DRZ significantly attenuates the cardiotoxicity induced by DXR and MTX, and that this protective activity can be assessed by morphological evaluation of cardiac tissues and by monitoring the concentrations of cTnT in serum.

Keywords Doxorubicin · Mitoxantrone · Dexrazoxane · Cardiotoxicity · Cardiac troponin T

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Introduction

Measurements of serum levels of cardiac troponin T (cTnT) have been used for the detection of myocardial damage from a variety of causes [4, 23, 24, 28, 29]. This method has been improved by the development of a specific and sensitive immunoassay that differentiates the cardiac from the skeletal muscle isoform of cTnT [22, 25, 30, 34]. There has been increasing interest in the use of cTnT as a biomarker of doxorubicin (DXR) cardiotoxicity. Initially, Fink et al. [9] found that concentrations of cTnT (determined by an early assay method) did not change in children given three to five doses of DXR. However, Ottlinger et al. [31] found that serum cTnT levels increase from nonmeasurable to low in children who receive DXR chemotherapy. Using an improved assay, Lipshultz et al. [27] observed that the small increases in serum concentration of cTnT in children after the first dose of DXR are predictive of subsequent risk for left ventricular dilatation and wall thinning. Seino et al. [35] observed increases in serum

cTnT levels in spontaneously hypertensive rats (SHR) given 1.5 mg/kg DXR weekly for 8 weeks. More recently, Herman et al. [19, 21] detected increases in serum levels of cTnT in SHR given 2 to 12 mg/kg DXR, and demonstrated a correlation between cTnT levels and the degree of myocardial damage in these animals.

The use of serum levels of cTnT to detect myocardial damage from other chemotherapeutic agents, such as mitoxantrone (MTX), has not been reported. Early studies indicate that MTX exerts significant antitumor activity [41] with minimal cardiotoxicity [13, 37, 39]. However, significant myocardial toxicity was identified in subsequent clinical and experimental studies [2, 14, 33, 40]. At clinically equivalent doses, this cardiotoxicity is considered to be less severe than that of DXR [1, 18, 32]. Compared to DXR, MTX causes similar myofibrillar loss, less-pronounced dilatation of the sarcoplasmic reticulum and more prominent mitochondrial alterations [18].

Both DXR and MTX form complexes with Fe(III) [18]. The DXR-iron complex is thought to facilitate the formation of toxic reactive oxygen species (ROS) in tissues [11]. Iron also may play a similar role in catalyzing the MTX-induced formation of cardiotoxic ROS. Dexrazoxane (DRZ), a bisdiketopiperazine, significantly attenuates DXR-induced cardiomyopathy [17, 38]. This cardioprotective activity is thought to be due to the conversion of DRZ to ADR-925, an intracellular iron chelator that can remove iron from the DXR-iron complex [6]. However, it is not clear whether DRZ is also capable of ameliorating MTX-induced cardiac damage [1, 26, 36]. The present study was initiated to compare, by monitoring serum cTnT concentrations and by morphological evaluation of cardiac tissue, the protective activity of DRZ against DXR- and MTX-induced cardiotoxicity. In addition, the study examined the ability of ADR-925 to remove Fe(III) from the MTX-Fe(III) complex.

Materials and methods

The experimental animals comprised 36 male SHR, 12 weeks of age, obtained from Charles River Breeding Laboratories (Wilmington, Mass.). The animals were housed individually and had access to rodent chow and water ad libitum. The experiment commenced after a 2-week acclimation period. DXR and DRZ were obtained from Pharmacia and Upjohn Laboratories (Columbus, Ohio) and MTX from American Cyanamid Company (Pearl River, N.Y.). All procedures performed during the course of the study were approved by the Center for Drug Evaluation and Research Institutional Animal Care and Use Committee and were in accord with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

The animals were divided into five groups (groups 1, 2, 4, 5, 6) of 5 animals each and one group (group 3) of 11 animals. Animals in groups 1 and 2 were given 1 mg/kg DXR (5.9 mg/m^2) via a tail vein at weekly intervals for up to 12 weeks. Animals in group 2 were pretreated with 25 mg/kg (147.5 mg/m^2) DRZ (i.p.) 30 min before DXR administration. Animals in groups 3 and 4 received 0.5 mg/kg MTX (3.0 mg/m^2) via a tail vein weekly for 12 weeks. This dose of MTX was used in our previous study [18]. The 11 animals in group 3 were dosed with MTX in two different subgroups that received the drug at different times. Animals in group 4 were pretreated with 25 mg/kg DZR 30 min prior to dosing with MTX. Animals in group 5 received 25 mg/kg DRZ (i.p.) followed 30 min later by an i.v. injection of saline. Animals in group 6 received i.p. and i.v. injections of comparable volumes of saline.

The animals were anesthetized with sodium pentobarbital 1 week after the 12th dose. Terminal blood samples were collected for determination of serum levels of cTnT, after which a complete necropsy was performed.

Pathological evaluation of the heart

Portions of the heart were fixed in phosphate-buffered 10% formalin, embedded in glycol methacrylate resin, sectioned at a thickness of 1 μm and stained with alkaline toluidine blue. The frequency and severity of DXR-induced myocardial lesions were assessed semiquantitatively by light microscopic examination without knowledge of the treatment [16, 17]. The alterations were scored on the basis of the number of myocytes showing myofibrillar loss and cytoplasmic vacuolization (score of 0 to 3 according to the method of Billingham [3]). Cardiac lesions in MTX-SHR were scored in a similar manner. Animals that died during the study and from which no terminal blood sample could be obtained were not included in the analysis of the data.

cTnT assay

To monitor serum levels of cTnT, blood samples were collected prior to the first dose (control) and after the sixth and ninth doses. In addition, terminal samples were obtained from moribund animals or 1 week after the 12th weekly dose of DXR or MTX. Blood samples were centrifuged and the serum was frozen at –40°C until assayed. Serum concentrations of cTnT were monitored by immunoassay (Elecsys STAT; Roche Diagnostics, Indianapolis, Ind.) without knowledge of the treatment.

Interactions of DRZ with MTX-Fe(III) reaction kinetics

The MTX used in this portion of the study was obtained by B.B.H. from Wyeth-Ayerst Canada, ADR-925 was obtained by B.B.H. from Pharmacia & Upjohn Laboratories (Columbus, Ohio), and EDTA, diethylenetriaminepentaacetic acid (DTPA) and desferrioxamine mesylate (DFO) were obtained from Sigma (St. Louis, Mo.). The MTX-Fe(III) complex was prepared at a 2:1 MTX to iron ratio under slightly acidic conditions (to prevent formation of insoluble ferric hydroxides) as previously described [18]. A small amount of the preformed MTX-Fe(III) complex was added to a thermostatted (25°C) 1-cm cell containing Tris/KCl (50 m*M*/150 m*M*, pH 7.4) buffer in a Cary 1 spectrophotometer and the solutions (50 μ*M* iron/100 μ*M* MTX) were allowed to equilibrate for about 10 min before the reaction was started by the addition of a small volume of chelator. The displacement of Fe(III) from the MTX-Fe(III) complex by the chelators was examined both by recording complete spectra (320 to 900 nm) at various time intervals after addition of the chelator and by following absorbance changes at 820 nm at which the absorbance of uncomplexed MTX is small compared to that of the complex [18].

Statistical analysis

The significance of differences in myocardial lesion scores among the various groups was determined by the Mann-Whitney test for nonparametric data. The Tukey-Kramer multiple comparisons test was used to assess the significance of differences among the groups in rat serum cTnT. $P < 0.05$ was taken as the level of significance.

Results

General toxicity and weight changes

Animals treated with DXR alone either died (two) or were killed (three) at a cumulative dose of 10 mg/kg (59 mg/m^2) DXR. One animal died after receiving the 12th dose of DRZ and MTX. Animals in all treatment groups gained weight during the initial 6 weeks of the study. The mean increase in body weight ranged from 14 g in the group receiving DRZ and DXR to 44 g in the saline-treated control animals. After the 6th dose, the body weight of animals given DXR began to decline, such that by both the 9th and the 10th doses it was significantly below that of the animals pretreated with DRZ and the control animals ($P < 0.01$). Animals given MTX alone lost an average of 12 g between the 9th and the 12th doses, but these animals still gained an average of 34 g over the course of the experiment. Animals given DRZ plus either MTX or DXR gained an average of 47 and 37 g, respectively, but weighed significantly less than the saline- or DRZ-treated control animals ($P < 0.05$). Control animals given DRZ or saline gained an average of 71 and 77 g, respectively, at the end of the 12-week experimental period (Fig. 1).

Gross anatomical changes

Excess pericardial and peritoneal fluid was observed in all five animals given DXR alone. Lesser amounts of fluids were noted in animals given the combination of DRZ and DXR. Evidence of fluid accumulation was also noted in three of the animals treated with MTX alone. Hemorrhage in the small intestine and cecum was visible in the animal that died after the 12th dose of DRZ and MTX. The kidneys from animals given MTX, DXR or DRZ and DXR appeared pale.

Myocardial pathology

All animals treated with MTX or DXR developed cardiac lesions that could be identified on light microscopic evaluation (Fig. 2). The characteristics of these lesions, cytoplasmic vacuolization and myofibrillar loss, have been observed previously in SHR [16, 17, 18] and other animal models as well as in human patients who had received DXR chemotherapy [7, 8]. Data on the incidence and severity of the myocardial lesions are summarized in Table 1. As mentioned previously, hearts from animals without available terminal blood samples (two treated with DXR, and one with DRZ and MTX) were excluded from this evaluation. Myocardial lesions (cardiomyopathy scores of 2.0, 2.5 and 3.0) were found in three animals killed after ten doses of DXR (59 mg/m^2). The severity of the myocardial lesions was significantly attenuated in animals treated with DRZ prior to the administration of DXR ($P < 0.05$; Table 1 and Fig. 2). In addition, all five animals in this group survived the entire 12-dose experimental regimen. All animals developed myocardial lesions following 12 doses of MTX (35.1 mg/m^2). Ten of these 11 animals had lesion scores of 2.0 (six animals) or 2.5 (four animals). Pretreatment of animals with DRZ prior to administration of MTX significantly attenuated the severity of the myocardial alterations (two animals 1.5 and two 1.0; $P < 0.05$). The hearts from control animals given either DRZ or saline alone were normal.

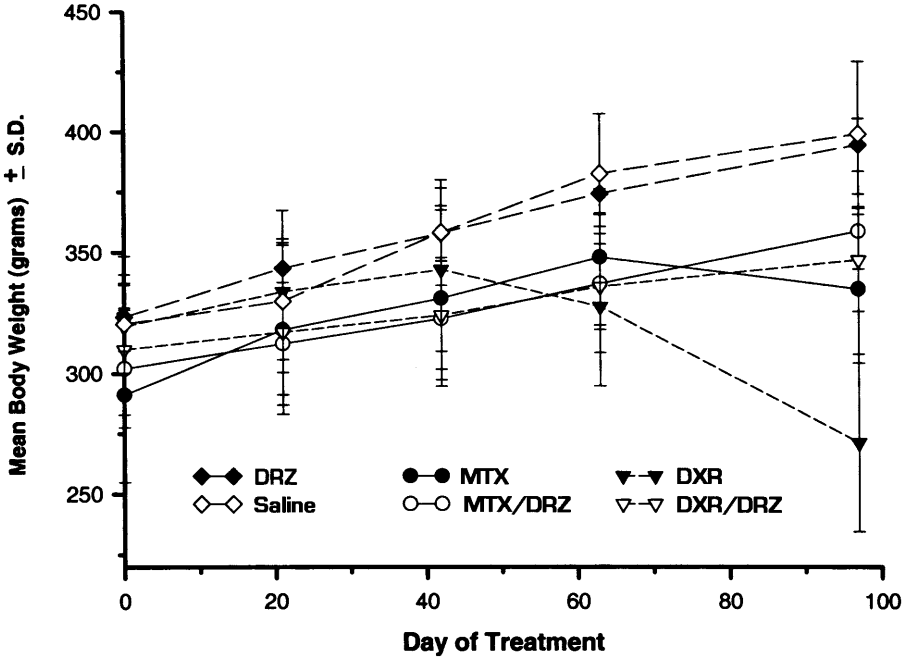

Fig. 1 Body weight changes in SHR given 1 mg/kg DXR, 25 mg/kg DRZ and 1 mg/kg DXR, 0.5 mg/kg MTX, 25 mg/kg DRZ and 0.5 mg/kg MTX, 25 mg/kg DRZ or saline weekly for 10–12 weeks. Each point represents the mean ± SD from three to five animals

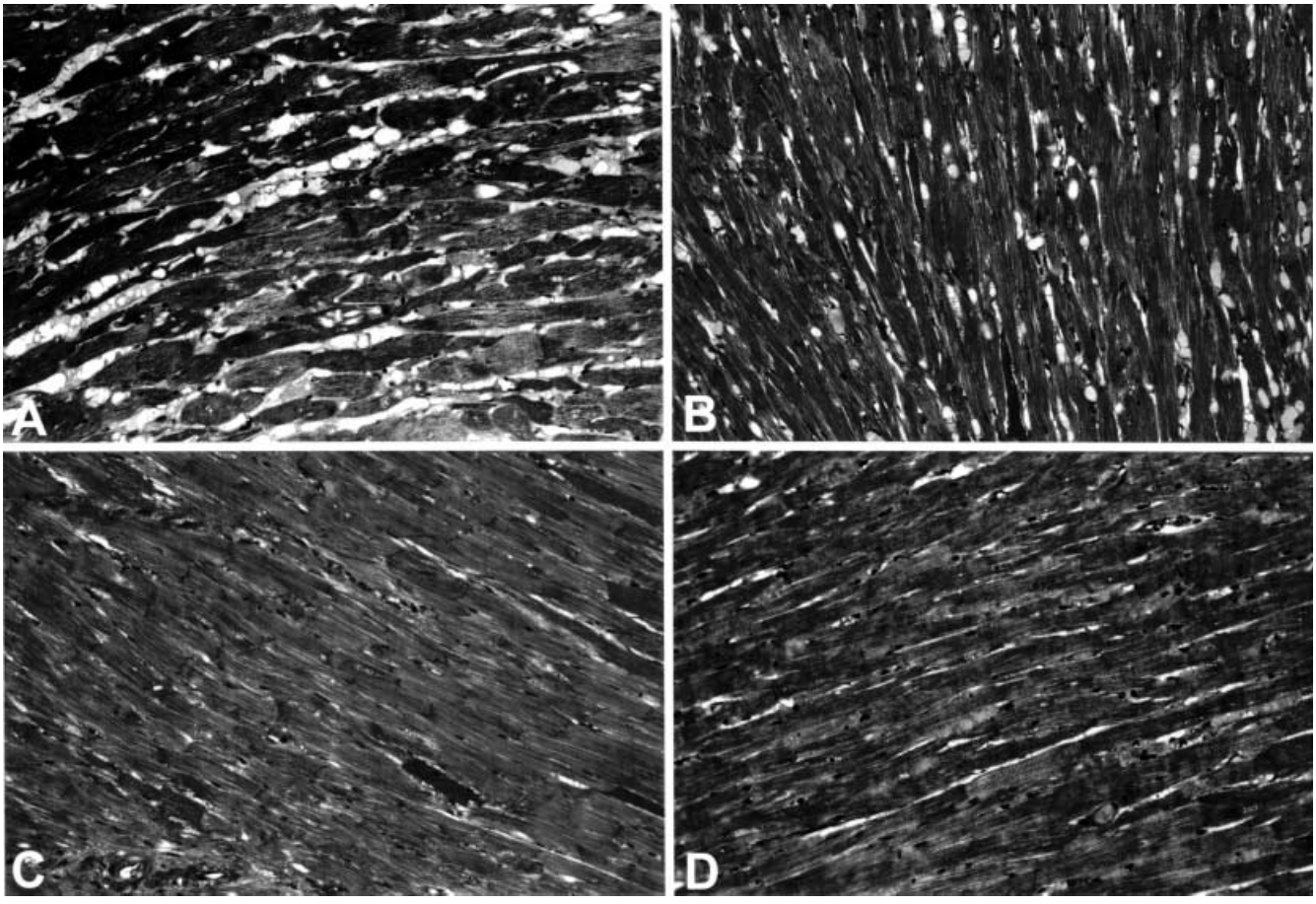

Fig. 2A–D Light micrographs showing cardiac lesions in glycol methacrylate-embedded, toluidine-blue-stained, 1 μm-thick sections of left ventricle of SHR. **A** Treatment with DXR induced severe lesions (cytoplasmic vacuoles and loss of myofibrils). **B** Treatment with 0.5 mg/kg MTX induced lesions that were similar in characteristics but less severe than those induced by DXR. **C** Pretreatment with 25 mg/kg DRZ prior to 1 mg/kg DXR resulted in minimal vacuolization. **D** Pretreatment with 25 mg/kg DRZ prior to 0.5 mg/kg MTX resulted in only a few small cytoplasmic vacuoles and minimal myofibrillar loss (each ×300)

Table 1 Cardiomyopathy scores in SHR treated with MTX or DXR with or without 25 mg/kg DRZ weekly for up to 12 weeks. Cardiomyopathy scores ranged from 0 to 3 according to the scoring method of Billingham [3]. Statistical comparisons were made using the Mann-Whitney test. *P*-values < 0.05 were accepted as significant

	Number of hearts	Cardiomyopathy score					
		0	1	1.5	2.0	2.5	3.0
DXR	3	0	0	0	1	1	1
DXR/DRZ[a]	5	0	1	3	1	0	0
MTX	11	0	0	1	6	4	0
MTX/DRZ[b]	4	0	2	2	0	0	0
DRZ	5	5	0	0	0	0	0
Saline	5	5	0	0	0	0	0

[a]Lesion scores significantly less severe than those from group given DXR alone ($P < 0.05$)
[b]Lesion scores significantly less severe than those from group given MTX alone ($P < 0.05$)

Serum levels of cardiac cTnT

Data on the serum levels of cTnT in animals in the various treatment groups are summarized in Fig. 3. Detectable amounts of cTnT were found in 5 of the 30 initial (pretreatment) control samples (0.03–0.12 ng/ml). However, cTnT could not be detected in the serum from four (two from the DXR group and one each from the DRZ and saline control groups) of these same five animals when a subsequent sample was obtained 6 weeks after initiation of dosing. Animals treated with DXR had average cTnT concentrations of 0.19 ± 0.08 and 0.79 ± 0.28 ng/ml after the 9th and 10th doses, respectively. The mean cTnT concentrations in serum of animals treated with DRZ prior to DXR were 0.02 ± 0.03 and 0.24 ± 0.13 ng/ml after 9th and 12th doses, respectively. These levels were significantly lower than those observed in the animals given DXR alone ($P < 0.05$). The lower cTnT levels in DRZ-pretreated animals also correlated with a significant reduction in the severity of the myocardial lesions.

After the 9th dose, only one animal in the group receiving MTX alone and one in the group treated with the DRZ/MTX combination had detectable amounts of cTnT (0.03 ng/ml and 0.01 ng/ml, respectively). After the 12th dose, all animals in these two groups had

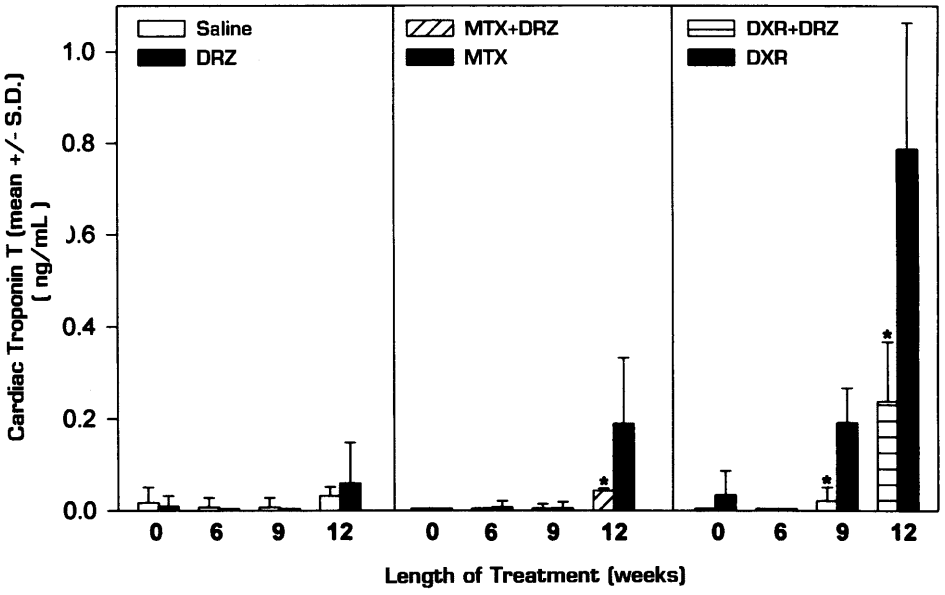

Fig. 3 Mean (±SD) serum levels of cardiac cTnT in SHR given either 0.5 mg/kg MTX or 1 mg/kg DXR with or without 25 mg/kg DRZ weekly for up to 12 weeks. *$P < 0.05$ vs groups receiving either MTX or DXR alone (Tukey-Kramer multiple comparison test)

elevations in serum cTnT concentrations. Pretreatment with DRZ also significantly attenuated the MTX-induced increase in mean serum levels of cTnT ($P < 0.05$). These decreases also correlated with a significant reduction in the cardiomyopathy score (from 2.0 to 1.25; $P < 0.05$). The mean serum cTnT levels after the 12th dose of MTX were significantly lower than after the 10th dose of DXR ($P < 0.05$). Cardiomyopathy scores were also less severe after the 12th dose of MTX (35.1 mg/m^2) than after the 10th dose of DXR (59.mg/m^2). These cumulative doses in the SHR are lower than those considered to cause significant cardiotoxicity in most human patients. At the end of the study, the mean cTnT concentrations in serum were 0.06 ± 0.09 ng/ml and 0.03 ± 0.02 ng/ml in DRZ and saline-treated controls, respectively. None of these animals had any detectable myocardial abnormalities.

Displacement of iron from its MTX-iron complex by ADR-925 and other chelators

The addition of various chelators to the MTX-Fe(III) complex resulted in spectral changes consistent with the displacement of Fe(III) from the complex (data not shown). We have previously shown that the MTX absorbance peak at 610 nm decreases and the absorbance in the 685–900 nm region greatly increases upon formation of the MTX-Fe(III) complex [26]. These spectral changes were substantially reversed to an uncomplexed MTX spectrum upon addition of the chelators. However, as shown in Fig. 4, this reversal was not totally complete, as the absorbance at 820 nm did not revert to that of the uncomplexed MTX (the absorbance of which is small compared to that of the complex) [26]. It has previously been noted that a small amount of dark precipitate develops upon formation of the MTX-Fe(III) complex [26]. Thus, the inability of the chelators to completely reverse these spectral changes is probably due to its failure to displace iron from this precipitate.

The displacement of Fe(III) from the MTX-Fe(III) complex was followed by a decrease in absorbance with time (Fig. 4a) at 820 nm. Both the degree and the rate of iron removal were dependent upon the concentration and the nature of the chelator. The strong iron chelators EDTA and DTPA resulted in the fastest and the most complete removal of Fe(III). At concentrations below 200 μM, all the chelators (except ADR-925) caused a nearly complete displacement of iron. The removal of iron from its complex with MTX was also studied as a function of the ADR-925 concentration. The absorbance-time traces were fitted to a three-parameter exponential decay equation to yield a pseudo-first-order rate constant k_{obs}. Small spectral changes with time were observed in the absence of any added ADR-925. These changes added to some of the uncertainty in obtaining accurate values of k_{obs}, which varied linearly with the concentration of ADR-925, yielding from the slope of the plot a second-order rate constant of 18 $M^{-1}\text{min}^{-1}$ (with an r^2 of 0.87 for an n of 7, data not shown). These results indicate that the reaction of ADR-925 with MTX-Fe(III) was a second-order reaction. The rate constant value is much lower than similarly calculated second-order rate constants for the reaction of ADR-925 with Fe(III) complexes of daunorubicin, DXR, epirubicin and idarubicin (11,500, 6900, 3900, and 4000 $M^{-1}\text{min}^{-1}$, respectively) [6]. In contrast, a limiting value of k_{obs} was observed for the anthracycline-Fe(III) complexes.

Discussion

The present study confirms that the serum concentrations of cTnT are increased in SHR given cumulative doses of 10 mg/kg DXR and shows for the first time that

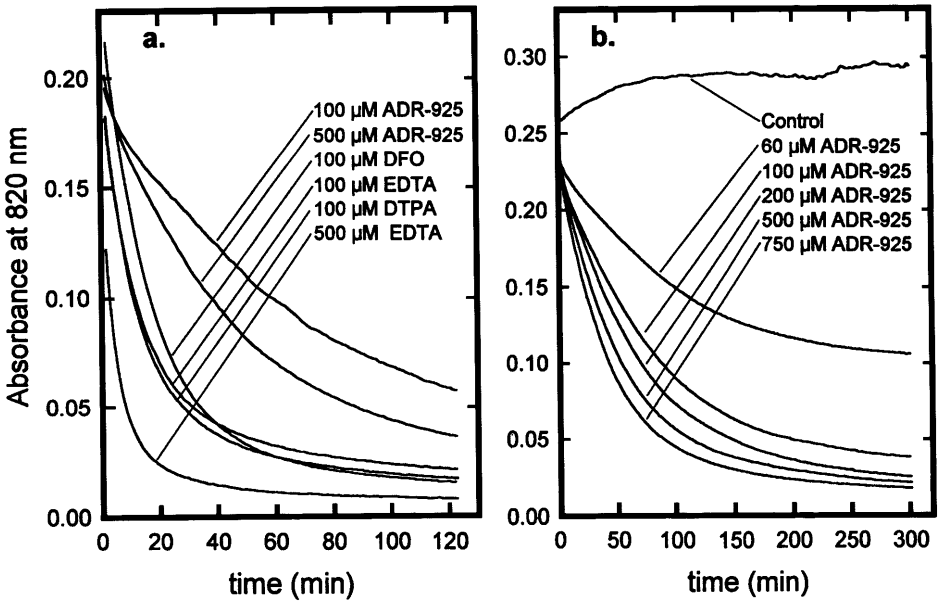

Fig. 4a, b Absorbance changes at 820 nm as a function of time for the reaction of (**a**) 100 μ*M* DTPA, 100 μ*M* or 500 μ*M* EDTA, 100 μ*M* DFO and 100 μ*M* or 500 μM ADR-925, and (**b**) 60, 100, 200, 500 or 750 μ*M* ADR-925 with the MTX-iron complex. The preformed MTX-iron complex (100 μ*M* MTX/50 μ*M* iron) was added to Tris/KCl buffer (pH 7.4) at 25°C and the chelators were then added to start the reaction

increased serum levels also occur after chronic administration of MTX (6 mg/kg). These cumulative doses resulted in significant clinical and morphological evidence of cardiotoxicity, which was similar to that observed in previous investigations [16, 17, 18]. The toxicity of both agents was attenuated by pretreatment with DRZ, and was accompanied by significant decreases in the magnitude of the elevations of the serum levels of cTnT.

DXR cardiotoxicity

The degree of cardiotoxicity induced by DXR in the present study is similar to that reported previously in SHR treated with cumulative doses of 10–11 mg/kg DXR [16, 17, 18, 21]. We have also demonstrated that the elevation in serum cTnT induced by DXR is dose-related [19, 21]. The concentrations of cTnT found in two previous studies ranged from 0.2 to 0.36 ng/ml (mean 0.28 ± 0.07 ng/ml) after cumulative doses of 10–12 mg/kg DXR [19] and 0.14 to 0.34 ng/ml (mean 0.24 ± 0.08 ng/ml) in SHR that had received 10 mg/kg DXR [21]. These elevations were associated with significant cardiomyopathy and with a decrease in immunohistochemical staining for cTnT in the hearts of these animals. In the present study we were able to monitor terminal serum cTnT levels in only three of the five DXR-treated animals and the values obtained (0.58, 0.68 and 1.1 ng/mg, mean 0.79 ± 0.28 ng/ml cTnT) were somewhat higher than those found in our previous studies using comparable cumulative doses of DXR [19, 21]. The reason for these differences is not known.

Several studies have shown that the morphological alterations induced in the heart by DXR are attenuated by pretreatment with DRZ [15]. The present study is the first to show that serum levels of cTnT are reduced (from 0.79 ± 0.28 ng/ml to 0.24 ± 0.13 ng/ml) in SHR given DRZ before DXR ($P < 0.05$). The lower cTnT concentrations were reflected in less-severe cardiomyopathy scores in the DRZ-pretreated SHR (mean of 1.5 compared to 2.5), even though these animals received all 12 doses of DXR.

MTX cardiotoxicity

The myocardial lesions observed in the present study after a cumulative dose of 6 mg/kg MTX were similar to those found in previous studies. This dose corresponded to twice the full dose of DXR tolerated by SHR [18]. As in the case of DXR-induced cardiomyopathy, these lesions were accompanied by a significant increase (vs control level) in the serum levels of cTnT (mean 0.20 ± 0.12 ng/ml; $P < 0.05$). DXR is thought to be capable of releasing both the free cytosolic and the bound myofibrillar pools of cTnT into the blood [21]. It is likely that MTX induces similar alterations in the cTnT pools. The serum concentrations of cTnT appeared to have a direct relationship with the severity of MTX-induced myocardial alterations, as shown by the cardiomyopathy scores. These observations show for the first time that the cardiotoxicity of MTX can be monitored using the serum levels of cTnT. Both the cardiomyopathy scores and the serum levels of cTnT found in the MTX-treated animals were significantly lower than those observed in DXR-treated animals ($P < 0.05$).

This is also the first report of the attenuation of MTX-induced cardiomyopathy by DRZ in SHR. Previous reports of the protective activity of DRZ against MTX-induced cardiotoxicity have been limited. DRZ has been found to provide significant cardioprotection (as determined by echocardiography) in two small groups of patients with relapsing myelogenous leukemia

who were being treated with additional daunorubicin and MTX [5, 26]. DRZ was also thought to be beneficial during induction with MTX in a patient with acute myelogenous leukemia and ischemic heart disease [42]. Earlier experimental studies could not clearly demonstrate protection against MTX-induced cardiotoxicity. In a mouse model, Alderton et al. [1] have found that DRZ protects against the cardiotoxicity of DXR and epirubicin, but not against that of MTX. Alterations induced by MTX in isolated neonatal rat heart myocytes are only partially attenuated by exposure to DRZ [36]. Coadministration of DRZ with MTX delays death in mice but does not prevent MTX-induced cardiotoxicity [36]. In the present study, DRZ attenuation of MTX cardiotoxicity was evident both in the cardiomyopathy scores (2.1 vs 1.3) and in the serum levels of cTnT (0.20 vs 0.04 ng/ml). With both DXR and MTX, treatment with DRZ resulted in a 40% decrease in mean cardiomyopathy scores and a three- to fourfold decrease in the mean serum levels of cTnT.

Iron complexes and the cardiotoxicity of DXR and MTZ

Our previous spectral studies have shown that both MTX and DXR form drug-iron complexes (2:1 with MTX and 3:1 with DXR) [18], which are thought to facilitate the generation of ROS capable of causing peroxidative damage to a variety of cellular components [10]. The fact that MTX, DXR and two analogues which are similar in structure to MTX and DXR, piroxantrone and losoxantrone, all have the capacity to bind iron suggests that the formation of a drug-iron complex may be a primary and common determinant of damage to cardiac tissues by these agents [20]. The similarity in morphological characteristics of the myocardial lesions observed in the hearts of animals treated with these four agents also suggests that a common pathogenetic mechanism of action is responsible for their toxicity. The present study showed that ADR-925 can remove iron from the MTX-iron complex. DRZ exerts significant cardioprotective activity against both MTX and DXR, in accord with the finding that this agent can remove iron from the iron complexes of both drugs.

The displacement of Fe(III) from its complex with MTX by ADR-925 occurred at a much slower rate ($t_{1/2}$ 51 min) than from the complexes with daunorubicin, DXR, epirubicin and idarubicin ($t_{1/2}$ 0.9, 1.6, 3.0 and 3.0 min, respectively) [6]. This observation suggests that Fe(III) forms a stronger complex with MTX than with the other agents just cited. As found in the present study with the MTX-Fe(III) complex, and reported previously, chelators such as EDTA and DTPA displace Fe(III) from its complex with DXR at a higher rate than does ADR-925 [12]. The latter agent has the ability to remove iron from the DXR-Fe(III) and the MTX-Fe(III) complexes. In both instances, DRZ decreased the cardiotoxicity induced by these agents. This protective activity was demonstrated by morphological evaluation of myocardial tissue and by monitoring serum concentrations of cTnT.

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