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

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Abstract Purpose: To compare the protective effect of dextrazoxane (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, diethylenetriaminepenta-acetic 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 · Dextrazoxane · Cardiotoxicity · Cardiac troponin T

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, Ottinger et al. [31] found that serum cTnT levels increase from nonmeasurable to low in children who receive DXR chemotherapy. Using an improved assay, Lipschultz 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. Dextrazoxane (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/m²) 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/m²) DRZ i.p. 30 min before DXR administration. Animals in groups 3 and 4 received 0.5 mg/kg MTX (3.0 mg/m²) 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 DZ 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, diethylenetriaminepentaaetic 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 mM/150 mM, pH 7.4) buffer in a Cary 1 spectrophotometer and the solutions (50 μM iron/100 μM 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.