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Advances in Brief

Use of Cardiac Troponin T Levels as an Indicator of Doxorubicin-induced Cardiotoxicity

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Abstract

The release of cardiac troponin T (cTnT) as a biomarker of doxorubicin-induced chronic cardiac injury was evaluated in the spontaneously hypertensive rat (SHR) model. Elevations in serum levels of cTnT and decreased immunohistochemical staining of heart sections for this protein were noted in SHRs treated with cumulative doses of doxorubicin (7 mg/kg) that induced only minimal histological alterations in myocytes. Concentrations of cTnT were further elevated, coincident with reduced immunohistochemical staining, in SHRs given 10–12 mg/kg doxorubicin.

Thus, monitoring serum levels of cTnT can detect doxorubicin-induced myocyte damage in SHR and may prove useful for the noninvasive evaluation of this toxicity in humans.

Introduction

The techniques (echocardiography and radionuclide ventriculography) used clinically for evaluating the cardiotoxicity induced by doxorubicin may be of limited value in detecting early myocardial damage (1, 2). One approach to this problem involves measurements of the serum concentrations of cardiospecific proteins that are released from damaged myocytes. Serum levels of cTnT[2] are of value in the diagnosis of myocardial infarction, acute myocarditis, and early myocardial damage in unstable angina (3). This study was initiated to explore the feasibility of using cTnT as a biomarker for detecting doxorubicin-induced myocardial damage in SHRs, which are a consistent and reproducible model for the study of the chronic cardiotoxicity of doxorubicin (4).

Materials and Methods

SHR Model. Twelve-week-old SHRs were obtained from the Charles River Breeding Laboratories (Wilmington, MA). All experimental procedures performed in this study were approved by the Center for Drug Evaluation and Research Institutional Animal Care and Use Committee. Nineteen SHRs were divided into two groups of 12 (group 1) and 7 (group 2) animals. SHRs in group 1 received 1 mg/kg doxorubicin (dissolved in saline; Pharmacia-Upjohn, Kalamazoo, MI) i.v. weekly for up to 12 weeks. This dose is known to induce a severe degree of cardiomyopathy in SHR (4). Animals in group 2 (controls) received 12 weekly i.v. injections of 1 ml/kg saline. The experiment was terminated, and complete necropsies were performed 1 week after injections 7–12. The frequency and the severity of the doxorubicin-induced myocardial lesions (myofibrillar loss and cytoplasmic vacuolization) were assessed semiquantitatively according to Billingham (5).

Serum levels of cTnT were measured prior to the initial dosing and after 2, 4, 7, and 10–12 doses of doxorubicin or saline using an ELISA (Enzyum-Test Cardiac T TnT; Boehringer Mannheim, Indianapolis, IN). Assay kits for cTnT were supplied by the manufacturer to N. R. and S. E. L.

Paraffin sections of formalin-fixed heart tissue were used to demonstrate the immunohistochemical localization of cTnT. The sections were treated according to the antigen retrieval microwave technique using Glyca Solution (BioGenex, San Ramon, CA). A mouse monoclonal antibody (clone T1/61; Serotec, Ltd., Oxford, England) directed against cTnT was used as the primary antibody; a horse antimouse IgG conjugated with FITC (Vector Laboratories, Burlingame, CA) was used as the secondary antibody. The sections were examined with a confocal microscope. All procedures were performed by investigators who had no knowledge of the drug treatment.

The Tukey-Kramer multiple comparisons test was used to evaluate the significance of the differences in cTnT concentrations in rat serum. $P \leq 0.05$ was taken as the level of significance.

Results

SHR Model

General Toxicity. Six SHRs that were given doxorubicin died after cumulative doses of 10–11 mg/kg. No terminal blood samples were available from these animals. Terminal blood samples were obtained from six SHRs euthanized after cumulative doses of 7–12 mg/kg. Saline-treated animals remained normal.

Myocardial Pathology. All animals given doxorubicin developed typical myocardial lesions. Myocyte necrosis was not observed in any of these animals. The SHRs given 10–12 doses of doxorubicin had Billingham scores of 3 (four animals), 2.5 (three animals), and 2 (three animals). The two SHRs that received seven doses of doxorubicin had only minimal cardiomyopathy (scores of 1 and 1.5). The hearts of SHRs given saline were normal.

Serum cTnT Levels. Pretreatment serum cTnT levels (n = 19) were 0.01 ± 0.01 ng/ml (range, 0.00–0.01 ng/ml). These levels remained relatively unchanged in animals given 2 mg/kg doxorubicin (0.01 ± 0.01 ng/ml; range, 0.00–0.02 ng/ml; n = 6) and 4 mg/kg doxorubicin (0.02 ± 0.02 ng/ml; range, 0.00–0.04 ng/ml; n = 5). A significant increase (0.09 ± 0.04 ng/ml) above the control cTnT level (0.01 ± 0.01 ng/ml) was observed in SHRs (n = 8) treated with 7 mg/kg doxorubicin. At this cumulative dose, small increases in cTnT (0.04 and 0.05 ng/ml) were detected in the two SHRs with minimal lesions (scores of 1 and 1.5; Table 1). Animals (n = 4) that received 10–12 mg/kg doxorubicin had serum levels of cTnT that ranged from 0.2 to 0.36 ng/ml (mean, 0.28 ± 0.07 ng/ml). The mean cTnT concentration found in these animals was significantly greater than that found in SHR after 7 mg/kg doxorubicin. The highest concentrations of cTnT (0.3 or 0.36 ng/ml) were detected in two SHRs with the most severe myocardial lesions (scores of 3; Table 1). cTnT concentrations in the saline-treated control SHRs (n = 7) were unchanged

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[2] The abbreviations used are: cTnT, cardiac troponin-T; SHR, spontaneously hypertensive rat.

Table 1 *Individual serum cTnT concentrations and corresponding myocardial lesion scores in SHRs treated weekly with 1 mg/kg doxorubicin for up to 12 weeks*

Treatment duration	cTnT (ng/ml)	Lesion score
7 weeks	0.05	1.5
	0.04	1.0
10–12 weeks	0.36	3
	0.20	2
	0.30	3
	0.25	2.5

during the course of the study (0.01 ± 0.01 ng/ml at both sampling times).

Immunohistochemical Staining for cTnT. The reaction for cTnT in control hearts was evenly distributed in the I bands of the myocytes (Fig. 1*A*). Other cardiac structures were unreactive. No staining was observed when the first antibody was omitted from the series of processing steps. Moderate but focal (Fig. 1*B*) decreases in the reaction were observed in the hearts of SHR treated with 7 mg/kg doxorubicin. SHRs treated with 10–12 mg/kg of doxorubicin showed a marked but still focal decrease in the intensity of the reaction. The cytoplasmic vacuoles present in many of the myocytes were negative (compare Fig. 1, *C* and *D*), and the staining of the I bands of the myofibrils was markedly reduced.

Discussion

Here, we show that the cardiac lesions produced in SHRs by the chronic administration of doxorubicin, in cumulative doses comparable to those used for cancer chemotherapy in human patients, can be monitored by following the serum levels of cTnT. In addition, immunohistochemical study demonstrated that the cardiac myocytes exhibiting the typical lesions of doxorubicin toxicity also have decreased staining for cTnT. These findings indicate that a loss of cTnT from damaged but nonnecrotic cells is the cause of the elevation in the serum levels of this protein.

In human myocytes (3), most of the cTnT (M_r 37,000) is present in the thin filaments of the myofibrils, and 6% is in a cytosolic precursor pool that is in equilibrium with the myofibrillar form. The cytosolic cTnT is thought to account for the very early release of this protein from ischemic myocytes (3); the myofilament-bound form is thought to account for the more delayed elevation of serum levels of this protein after infarction (3).

The loss of cTnT from myocytes would appear to be related to the myofibrillar lysis and the plasma membrane alterations produced by doxorubicin (6). The myofibrillar lysis can be regarded as the result of several coexisting processes, including inhibition of protein synthesis, decreased expression of muscle-specific proteins, inadequate assembly of sarcomeres, and increased degradation of myofibrillar components. Structural-functional studies have shown a correlation between the extent of morphological changes and that of cardiac dysfunction in doxorubicin cardiotoxicity (5). However, experience with measurements of cTnT in doxorubicin cardiotoxicity has been very limited. Fink *et al.* (7) reported that the serum levels of cTnT were not increased in children who had received three to five courses of anthracycline chemotherapy. In other children receiving doxorubicin therapy, Ottlinger *et al.* (8) found that the serum levels of cTnT increased from nonmeasureable to very low. Also in children, Lipshultz *et al.* (9) reported that low level increases in cTnT after the initial dose of doxorubicin were predictive of subsequent risk for myocardial injury. Seino *et al.* (10) found elevated serum concentrations of cTnT in SHRs receiving eight weekly doses of 1.5 mg/kg doxorubicin. Direct comparisons of these data with those obtained here are not possible because of the numerous changes that have been

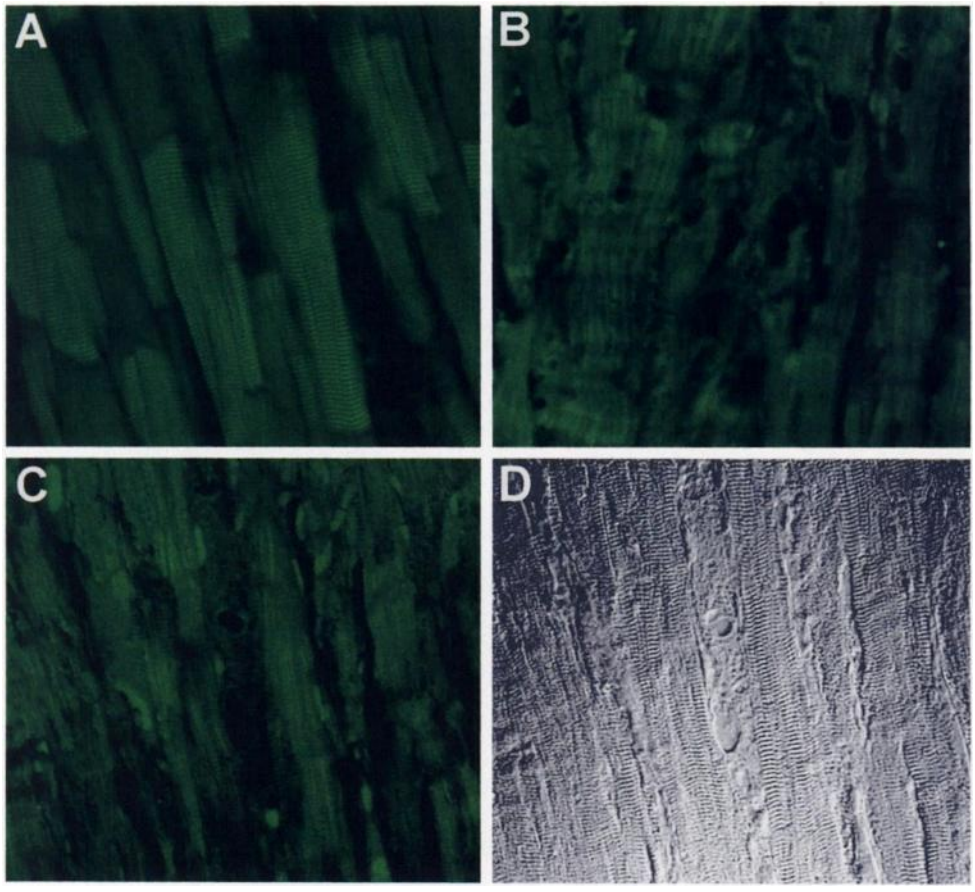

Fig. 1. Immunostaining for cTnT in sections of heart of SHR. *A–C*, reaction (green fluorescence) for cTnT. *D*, structural features of the tissue illustrated in *C*. *Magnification* (*A–D*), ×400. *A*, normal reaction (green fluorescence), which is homogeneously distributed throughout the cytoplasm of the myocytes from a control heart. *B*, after treatment with 7 mg/kg doxorubicin, there is a mild and focal decrease in the intensity of the staining. *C*, after treatment with 12 mg/kg doxorubicin, there is a more pronounced but still focal decrease in the intensity of the staining. *D*, same area as shown in *C*, but viewed using Nomarski differential interference contrast optics to demonstrate marked vacuolization and loss of contractile elements in some myocytes.

made during the past several years in the methods used for the assay of cTnT. Very recently, serum levels of cardiac troponin-I have been used to monitor doxorubicin cardiotoxicity in patients (11). Here, increased serum concentrations of cTnT occurred at a point (7 mg/kg doxorubicin) at which there was only minimal myocyte damage (Billingham scores of 1 and 1.5). These observations point to the feasibility of using serum concentrations of cTnT to detect early cardiac injury induced by anthracycline chemotherapy.

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