Cancer Chemother Pharmacol (2003) 52: 431–434
DOI 10.1007/s00280-003-0675-z

SHORT COMMUNICATION

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Cardiac troponin T as an indicator of reduced left ventricular contractility in experimental anthracycline-induced cardiomyopathy

Received: 27 March 2003 / Accepted: 4 June 2003 / Published online: 9 July 2003

Abstract *Purpose*: Cardiac troponin T (cTnT) plasma concentration is considered a useful marker of anthracycline-induced cardiomyopathy. In this study we used daunorubicin-treated Chinchilla rabbits as a model to investigate the relationship between left ventricular contractility and cTnT plasma concentrations. *Methods*: Two groups of animals were used: a control group ($n = 8$) received i.v. saline, and an experimental group ($n = 11$) received daunorubicin (3 mg/kg, i.v.). The substances were administered once weekly for 10 weeks, and 5–7 days after the last administration, left ventricular cardiac contractility (dP/dt_{max}) was invasively measured as a contractility index and blood was sampled for cTnT concentration determination (Elecsys Troponin T STAT immunoassay). *Results*: Cardiac contractility was significantly lower in seven surviving daunorubicin-treated animals than in control animals (745.7 ± 69.3 vs 1393.4 ± 25.5 kPa/s; $P < 0.001$), while cTnT plasma concentrations were significantly increased (medians 0.278 vs 0.000 ng/ml; $P < 0.001$). When the dP/dt_{max} values of individual daunorubicin-treated animals were plotted against the corresponding cTnT plasma concentrations, a close negative linear correlation was found ($R = -0.910$; $P < 0.005$; regression equation: $dP/dt_{max} = -1861 * cTnT + 1234$). *Conclusions*: This study suggests that determination of cTnT plasma levels, which is simple and inexpensive, could be used in anthracycline-treated patients for left ventricular systolic function assessment and contractility estimation.

Keywords Daunorubicin · Cardiotoxicity · Left ventricular contractility · Cardiac troponin T

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Introduction

Anthracycline antibiotics (e.g. doxorubicin and daunorubicin) are among the most effective agents in the treatment of various malignancies. Their clinical usefulness is unfortunately limited by dose-dependent cardiotoxicity. The highest risk is associated with chronic administration, when severe cardiomyopathy and congestive heart failure may develop [10, 15]. Accurate monitoring of cardiac function is therefore essential both during and after the administration of anthracycline-containing chemotherapy. Recently, cardiac troponins T and I (cTnT, cTnI) have been reported to be very sensitive markers of myocardial injury from a variety of causes, including the administration of anthracyclines, both in humans [3, 14] and in animal models [5, 7, 9].

In the rabbit, chronic administration of anthracyclines causes reproducible cardiac damage, similar to that observed in humans, and the rabbit is therefore considered a useful animal for experimental anthracycline cardiomyopathy induction [6]. The results of our previous study have shown that cTnT plasma concentrations gradually increase with repeated daunorubicin administrations, and may thus serve as a marker of daunorubicin-induced cardiomyopathy in rabbits [1].

The aim of the present study was to experimentally induce daunorubicin cardiomyopathy, and then to explore the relationship between left ventricular contractility and cTnT plasma concentrations. We also addressed the question as to whether the cTnT measurements could be used for left ventricular systolic function estimation.

Material and methods

The study included 19 Chinchilla male rabbits with an average body weight of 3.3 kg at the beginning of the experiment. The study was performed under the supervision of the Ethical Committee of Charles University in Prague, Faculty of Medicine in Hradec Králové, and complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1985).

Under ketamine anaesthesia (Calypsol inj., 50 mg/kg i.m.; Gedeon Richter, Hungary), daunorubicin (Cèrubidine, 3 mg/kg; Bellon Rhône-Poulenc Rorer, France) was administered to the marginal ear vein of 11 animals once weekly for 10 weeks. Control rabbits ($n = 8$) received saline (1 ml/kg, i.v.).

Invasive contractility measurements were made 5–7 days after the last administration using an ADI PowerLab/8SP instrument (Adinstruments, Castle Hill, NSW, Australia). Under pentobarbitone anaesthesia (Nembutal Sodium inj., 30 mg/kg i.v.; Abbott, North Chicago, Ill.), a catheter was inserted via the left carotid artery into the left heart ventricle. The maximal rate (i.e. the maximum of the first derivative) of the pressure rise in the isovolumetric phase of systole (dP/dt_{max}) was determined as an index of the left ventricular contractile function. Immediately after dP/dt_{max} measurement, blood was sampled for cTnT concentration determination, and the animals were killed. The concentrations of cTnT in heparinized plasma samples were measured using the Elecsys Troponin T STAT immunoassay on an Elecsys 2010 analyzer (Roche, Basel, Switzerland) with a detection limit < 0.010 ng/ml. Values below the detection limit were considered to be zero.

Statistical analysis

The statistical software SigmaStat for Windows 2.0 (Jandel, Erkrath, Germany) was used in this study. Significances of the differences between the daunorubicin and control groups were estimated using the *t*-test (dP/dt_{max}) and Mann-Whitney Rank Sum test (cTnT). Pearson correlation coefficients and linear regression analysis were used to describe the relationship between cTnT plasma concentrations and left ventricular dP/dt_{max}. Grubbs' test was used for the detection of outlying values.

Results

Of 11 animals in the daunorubicin group, 4 died prematurely with signs of left heart failure (hydrothorax and hydropericardium), all of them in the 10th week. The measurements were thus performed on seven daunorubicin-treated rabbits. In the control group, no premature deaths occurred.

As seen in Fig. 1, left ventricular contractility (dP/dt_{max}) in the daunorubicin-treated group was clearly and significantly reduced to 54% of the control group values. Plasma concentrations of cTnT in the control animals were very low, the highest concentration obtained being 0.024 ng/ml. On the other hand, in the daunorubicin-treated group, cTnT levels were markedly increased, ranging from 0.109 to 0.395 ng/ml (Fig. 2).

When the dP/dt_{max} values obtained in individual daunorubicin-treated animals were plotted against the corresponding cTnT plasma concentrations, a close negative correlation was found (Fig. 3). The following linear regression equation was calculated:

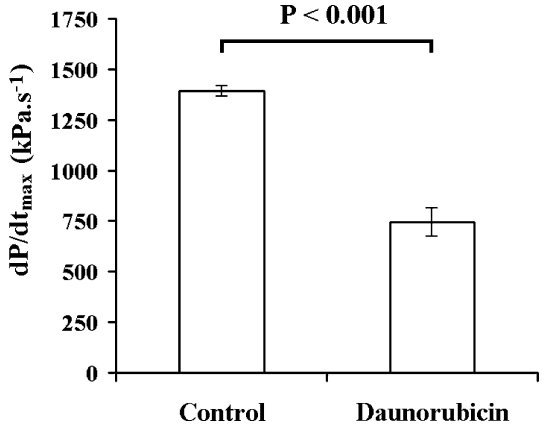

Fig. 1 Left ventricular contractility following ten i.v. administrations of daunorubicin (3 mg/kg weekly) or saline (*control*). The data are presented as means ± SEM

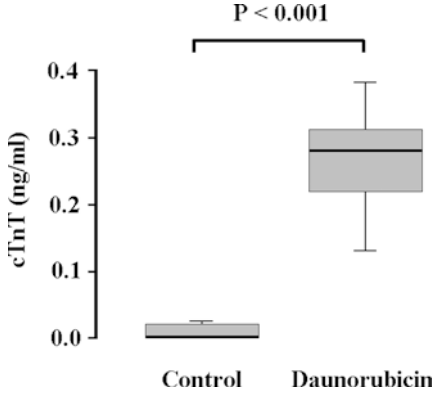

Fig. 2 Plasma concentrations of cTnT following ten i.v. administrations of daunorubicin (3 mg/kg weekly) or saline (medians, 10th, 25th, 75th and 90th percentiles)

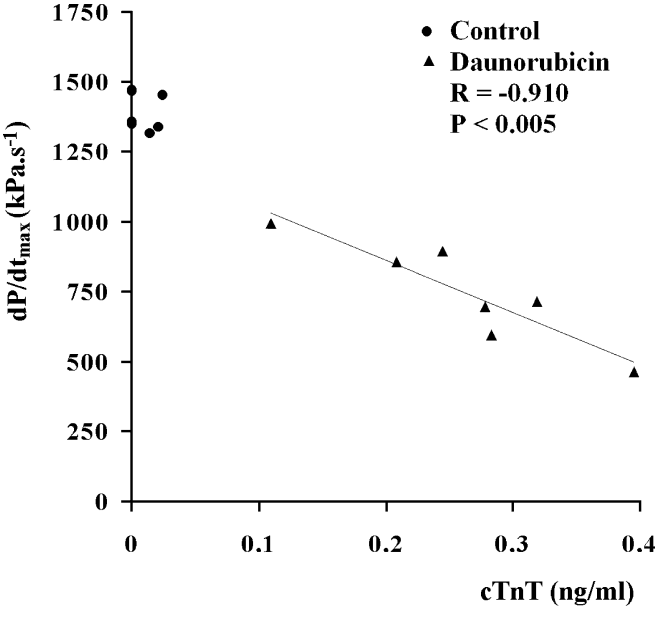

Fig. 3 Scatterplot of the left ventricular contractility versus cTnT plasma concentrations with the linear regression line

$$dP/dt_{\max}(kPa.s^{-1}) = -1861 \pm 380 \times cTnT(ng/ml) + 1234 \pm 105$$

The absolute member of this equation, i.e. the point where the regression line crosses the ordinate (cTnT = 0), was close to the control group contractility value (1234 ± 105 vs 1393 ± 26 kPa/s).

Discussion

Cardiac troponin T is a very sensitive marker of myocardial damage. Its determination was originally introduced for myocardial infarction detection [12], and in fact cardiac troponins have now replaced CK-MB as the standard for myocardial infarction laboratory diagnosis [11]. The utility of troponins in the assessment of heart failure of non-ischaemic origin has also been suggested [13] and cardiac troponins are now becoming recognized as powerful biomarkers of chemotherapy-induced cardiomyopathy, especially as they seem to be able to identify patients with latent myocardial damage and those who are at increased risk of cardiac events [3]. According to a new hypothesis, the release of troponin and/or troponin degradation products is not only specific for necrotic tissue, but it may occur from viable damaged cardiomyocytes as well. Troponins have been shown to be the targets of neutral Ca^{2+}-activated protease I (calpain I). This enzyme is activated in the presence of elevated intracellular Ca^{2+} concentrations, which occur during different pathological states (including anthracycline cardiotoxicity). Activated calpain I degrades troponins, and this leads to impaired interaction between actin and myosin and thereby to deterioration of myocardial contractile function [16].

The relationship between the plasma levels of troponins and other indices of anthracycline-induced heart impairment is the subject of current investigation. Herman et al. [8, 9] have reported that in spontaneously hypertensive rats treated with doxorubicin or mitoxantrone, cTnT levels closely parallel the severity of myocardial morphological lesions. Similarly, Bertinchant et al. [2] have recently shown in doxorubicin-treated rats that increased cTnT (but not cTnI) concentrations are correlated with an increase in left ventricular diameters as well as with the severity of myocardial morphological changes. In a clinical study [3], a correlation between cTnI and a decrease in left ventricular ejection fraction was found in a cTnI-positive subgroup of patients treated with a high-dose chemotherapy.

Our present experimental study confirmed that repeated 10-week i.v. daunorubicin administration at a cumulative dose of 30 mg/kg causes in rabbits pronounced impairment of left ventricular systolic function, as indicated by a significant dP/dt_{max} reduction. Even though the direct invasive haemodynamic measurement of left ventricular function is exact and reliable [4], its routine use is limited by its invasiveness, and the complicated and time-consuming nature of the procedure. On the other hand, the measurement of cTnT plasma concentration is a simple, noninvasive and low-cost.

For the first time in a study dealing with anthracycline-induced cardiomyopathy, increased cTnT plasma concentrations were correlated with parallel determinations of changes in dP/dt_{max}. Our finding that cTnT is strongly negatively correlated with contractility in a linear manner seems to be promising. With the obtained regression equation, any value of cTnT plasma concentration ranging from 0 to 0.395 ng/ml can be used in this model for dP/dt_{max} estimation.

In conclusion, the present study confirmed cTnT plasma concentration as a useful indicator of anthracycline-induced cardiomyopathy. Our results also suggest the feasibility of the use of cTnT in anthracycline-treated patients, not merely as a cardiac risk marker, but also for left ventricular contractility assessment. In future experimental and clinical studies, it is necessary to further evaluate the potential of troponins as cardiac contractility predictors using other anthracycline-treatment protocols, and with a wider range of dosages and higher numbers of experimental subjects.

Acknowledgements The authors would like to thank Mrs. Ludmila Koželuhová and Mrs. Jiřina Hoffmanová for their skilful technical assistance during the study, and Assoc. Prof. Bohuslav Mánek for his kind revision of the English text. This study was supported by Grant GA CR no. 305/03/1511, and by Research Projects MSM 111500002 and CEZ J13/98:11600002.

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