

TROPONINS IN EXPERIMENTAL STUDIES

Michaela Adamcová¹, Vladimír Geršl², Jarmila Macháčková², Radomír Hrdina³, Ivona Klímtová³, Tomáš Šimůnek³, Jaroslava Vávrová⁴, Josef Bukáč⁵

Charles University in Prague, Faculty of Medicine in Hradec Králové: Department of Physiology¹, Department of Pharmacology², Department of Biophysics⁵; Charles University in Prague, Faculty of Pharmacy in Hradec Králové: Department of Pharmacology and Toxicology³; University Hospital in Hradec Králové: Institute of Clinical Biochemistry and Diagnosis⁴

Summary: The aim of our study was to compare the diagnostic performance of cardiac troponin T (cTnT) and cardiac troponin I (cTnI) in three groups of rabbits: 1) control (saline 1 ml/kg i.v.); 2) daunorubicin (3 mg/kg i.v.); 3) daunorubicin (3 mg/kg i.v.) + dexrazoxane (60 mg/kg i.p.). The drugs were given once a week, 10 administrations. The concentration of cTnT was measured using Elecsys Troponin T STAT Immunoassay (Roche). The concentration of cTnI was measured using AxSYM Troponin I (Abbott). The linear regression model was applied to see if there is a dependence between cTnT and cTnI. The coefficient of determination ($R^2 = 0.79$) was acceptable only in the control group. In the remaining cases (i.e. in the daunorubicin group and in the daunorubicin + dexrazoxane treated group) R^2 was too small (0.53, and 0.06). We may conclude that in rabbits after repeated administration of cardiotoxic or cardioprotective drugs meaningful dependence between cTnT and cTnI was not found. The choice of the most suitable cardiomarker in laboratory animals deserves further studies.

Key words: Cardiac troponin T; Cardiac troponin I; Dexrazoxane; Cardiomyopathy; Rabbits

Introduction

The cardiotoxic effects belong among the most serious adverse effects of some antineoplastic drugs. These effects often limit the use of these antitumour drugs or may be the cause of severe, even lethal, therapeutic complications. In the antineoplastic therapy with anthracycline derivatives, such as doxorubicin and daunorubicin, three distinct types of cardiotoxicity have been described. First, acute or sub-acute injury; second, chronic cardiotoxicity resulting in cardiomyopathy, which is clinically most important; and third, late-onset ventricular dysfunction (4). The development of cardiomyopathy (changes in ECG, myocardial fibrosis and necrosis, cardiomegaly and chronic congestive cardiomyopathy) is closely connected with the total cumulative dose of anthracyclines (19).

We mostly assume that cardiotoxicity of anthracyclines is caused by oxygen-free radicals (15). Many drugs have been studied with the aim to prevent or reduce cardiomyopathy. At present, the only relatively effective drug is the derivative of EDTA - dexrazoxane (11,20,21). The mechanism of the cardioprotective activity of dexrazoxane (ICRF-187) is connected with intracellular chelation of the ions, including Fe ions, which results in slowing down the formation of free oxygen radicals induced with anthracyclines

(12). Although various methods have been used to predict congestive heart failure in anthracycline cardiomyopathy, none of them is sufficient (9).

The long-term intravenous administration of the doxorubicin or daunorubicin in rabbit induce the signs of cardiotoxicity (6,7) and the rabbits model is thus considered to be a satisfactory animal model for anthracycline-induced cardiomyopathy (5,18). The doses about 40 and more mg/m² (administered once weekly) are used to induce cardiomyopathy in rabbits (8). The results of our previous studies showed that cardiac troponin T may be a useful predictive marker of daunorubicin-induced cardiomyopathy in rabbits (1,2). The aim of this paper was to compare the diagnostic performance of cardiac troponin I (cTnI) and cardiac troponin T (cTnT) for the evaluation of cardiotoxic (and possibly, cardioprotective) effects of new drugs in experimental studies.

Methods

All experiments used in this study were approved by the Ethical Committee of the Charles University, Faculty of Medicine in Hradec Králové.

Medium size Chinchilla male rabbits of average weight 3 kg at the beginning of the experiment served as experi-

mental animals. The study was carried out on three groups of animals:

- control (saline 1 ml/kg i.v.), once a week, 10 administrations, 3 rabbits
- daunorubicin (Cérubidine, Bellon Rhone-Poulenc, France, 3 mg/kg i.v.), once a week, 10 administrations, 4 rabbits
- daunorubicin (Cérubidine, Bellon Rhone-Poulenc, France, 3 mg/kg i.v.) + dexrazoxane (Cardioxane, Chiron, Netherlands, 60 mg/kg i.p.), once a week, 10 administrations, 5 rabbits

The venipunctures for biochemical examination were performed in the selected time intervals during the whole experiment (before and 24 hrs after the 1st, 5th, 8th, and 10th administration and at the end of the experiment).

The concentration of cTnT in heparinized plasma samples was measured using Elecsys Troponin T STAT Immunoassay (Roche) on the Elecsys 2010 immunoassay analyser (Roche). The concentration of cTnI in heparinized plasma samples was measured using AxSYM Troponin I (Abbott) on the analyser AxSYM (Abbott).

Statistical evaluation was performed with the NCSS software.

Results

We have studied relationship between cTnT (independent variable x) and cTnI (dependent variable Y) using linear regression in the form $Y = \alpha + \beta x + \epsilon$. First we have tested the hypothesis that $\beta = 0$ which would mean that Y is independent of x . We have found out that hypothesis of independence was accepted only in the group that received daunorubicin and dexrazoxane repeatedly. Further, the strength of dependence was measured with the coefficient of determination R^2 . R^2 was acceptable only in the control group. R^2 was too small in the remaining cases. It means that such a model is not suitable for prediction. The data are shown in the following figures; statistical characteristics are given in Table 1.

Tab. 1: Simple linear regression of cTnI depending on cTnT.

Group	n	$H_0: \beta = 0$ p - value	R^2
Control	27	0.0000	0.79
Daunorubicin	34	0.0001	0.53
Daunorubicin + dexrazoxane	45	0.1227	0.06

$H_0: \beta = 0$ - cTnI is independent of cTnT

R^2 - coefficient of determination

Discussion

Troponin is a thin-myofilament associated regulatory complex of the myocyte that controls the interaction of

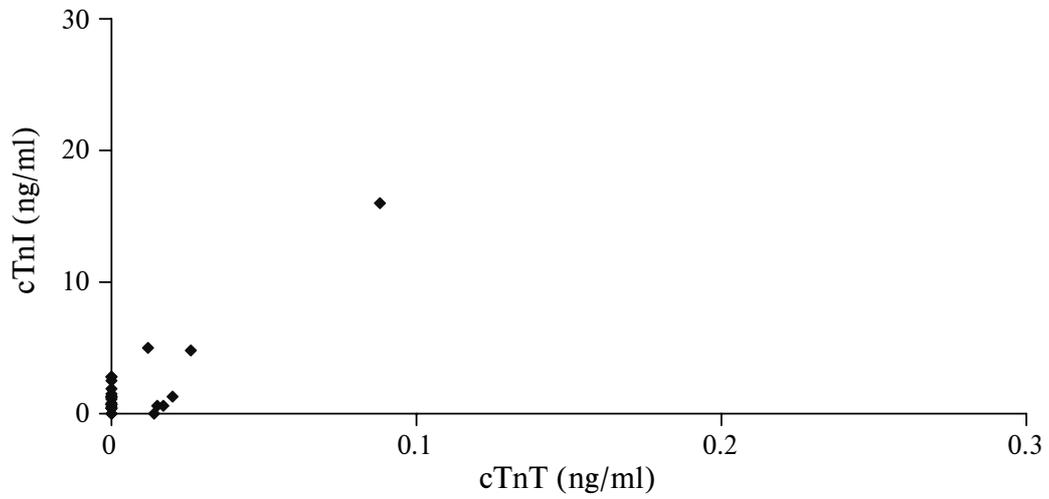
myosin and actin in response to alterations in intracellular Ca^{2+} concentrations. The troponin complex consists of three subunits called according their function: troponin T (TnT) - binds the complex to tropomyosin, troponin C (TnC) - binds divalent calcium ions and then undergoes conformational changes inducing progressive change in troponin I (TnI) that inhibits actin and myosin interaction in the absence of calcium. TnI and TnT exist in three different isoforms with a unique structure that is encoded by three different genes: one for slow-twitch skeletal muscle, one for fast-twitch skeletal muscle and one for cardiac muscle (13). Both cTnT and cTnI are considered to be reliable biomarkers with sufficient sensitivity and specificity for cardiac injury in the majority of laboratory animals (3). There is a great sequence homology in cTnT and cTnI among different species, certainly in higher vertebrates (16,17), which enables us to use clinical assays for both cardiac troponins in experimental medicine.

In our study we measured serum concentration of both cardiomarkers in more than 100 samples obtained from rabbits under various conditions. A surprisingly strong dependence of cTnI on cTnT was found only in the control group where the values were close to zero. Under physiological conditions troponins are almost absent in blood because of their zero turnover. The group with repeated administration of daunorubicin and the one with daunorubicin combined with dexrazoxane had the coefficients of determination too small to be considered acceptable.

We believe that the explanation of these results could rather be found in different sensitivity or specificity of these markers. Even though the kinetics of both troponins are very similar, some of their characteristics are not identical. First, they differ in intracellular compartmentation. The majority of troponins is myofibril bound, the minority is found as a soluble cytoplasmic pool, which probably serves as a precursor pool for the synthesis of troponin complex. The cytosolic fraction of cTnT in human myocardium is estimated to be 6 % of total TnT, this fraction is only 2.8 % for cTnI, but it is necessary to take into account that the marker proteins differ in their distribution from one species to another. The biological half-life of cTnT is 120 min, but only 90 min for cTnI. The molecular weight of cTnT is 37-39 kDa, the molecular weight of cTnI is 24 - 26.5 kDa (13).

Furthermore, while only one commercial assay is currently available for cTnT, a number of cTnI assays are available. There exist differences between assays in the epitopes on the cTnI molecule recognised by the anti-cTnI antibody and it should be noted that the tissue reactivity will likely vary as a function of the homology of tissue troponin recognised by different cTnI immunoassays that are commercially available (3).

On the other hand, it is interesting that a recent study (14) gave evidence that the predominant form of cTnI in the circulation is associated with troponin subunits cTnC and cTnT. The presence of the troponin complex in the se-



rum suggests that assays that measure TnI or TnT are in fact measuring the same circulating complex with different levels of detection.

To sum up, no meaningful dependence of cTnI on cTnT (with the exception of control group) has been found in rabbits. Although the choice of the best biochemical marker of myocardial damage does not seem to be simple and may differ from one species to another, further studies are needed because there is an increasing interest in the specific detection of myocardial damage in pre-clinical experiments (10).

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MUDr. Michaela Adamcová, Ph.D.,
Charles University in Prague,
Faculty of Medicine in Hradec Králové,
Department of Physiology,
Šimkova 870, 500 01 Hradec Králové,
Czech Republic.
e-mail: adamcova@lfhk.cuni.cz