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Troponin-T and Brain Natriuretic Peptide as Predictors for Adriamycin-Induced Cardiomyopathy in Rats

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Background Clinical methods for the early detection of doxorubicine (adriamycin; ADR) -induced cardiotoxicity have not been established. This study prospectively investigated whether atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and cardiac troponin T (TnT) are predictors for ADR-induced cardiotoxicity, and examined the correlations between the serum concentrations of these biomarkers and the functional alternations associated with ADR-induced myocardial damage.

Methods and Results Male Wistar rats were injected weekly with 2 mg/kg of ADR via the tail vein for 8 weeks to induce cardiotoxicity. Echocardiograms of each ether anesthetized rat were taken at 6, 8, 10 and 12 weeks after the first administration of ADR, and blood samples collected from the tail vein were used to quantify plasma ANP and BNP, and serum TnT after echocardiography. Plasma BNP and serum TnT significantly increased from 6 to 12 weeks (81.5 to 173.3 pg/ml ($p<0.001$), <0.01 to 1.09 ng/ml ($p<0.05$), respectively) with deterioration of left ventricular % fractional shortening (%FS) (58.6% to 36.8%). The %FS significantly correlated with TnT ($r=–0.51$, $p<0.001$) and BNP ($r=–0.75$, $p<0.0001$); however, the increase of TnT was antecedent to the increase of BNP and the deterioration of %FS.

Conclusion Plasma BNP and serum TnT concentrations, especially TnT, measured by this highly sensitive method are useful predictors for ADR-induced cardiomyopathy. (*Circ J* 2004; **68:** 163 – 167)

Key Words: Adriamycin; Cardiomyopathy; Natriuretic peptides; Predictor; Troponin T

Doxorubicin (adriamycin; ADR) remains one of the most effective and useful antineoplastic agents for a variety of malignancies, but its practical therapeutic use is limited by cumulative dose-related cardiotoxicity, resulting in dilated cardiomyopathy (DCM) and fatal congestive heart failure (CHF)[1,2] The occurrence of CHF is unpredictable and a useful clinical predictor for the progression of left ventricular (LV) dysfunction caused by ADR is needed. Clinically, serial measurement of the left ventricular ejection fraction (LVEF) by echocardiography or radionuclide ventriculography is available;[3,4] but the specificity and accuracy of either method are not satisfactory. The exact pathophysiological mechanisms leading to the development of cardiomyopathy are unclear, but we have demonstrated that apoptosis is involved in ADR-induced cardiomyopathy (ADR-CM) in rats and occurred through a Fas-dependent pathway[5,6] Our ADR-CM model rat showed histological changes similar to those in human ADR-CM; that is, degeneration and loss of myocytes, compensatory hypertrophy of residual myocytes, and interstitial fibrosis[7] It also showed both histologically and functionally that the number of apoptotic myocardial cells increased with the progression of ADR-CM.

Natriuretic peptides are secreted from the heart in response to atrial or ventricular overload[8,9] Atrial natriuretic peptide (ANP) is secreted mainly from the atria and brain natriuretic peptide (BNP) from the ventricle. Although both ANP and BNP increase in congestive heart failure, BNP is more sensitive and specific than ANP. Some researchers report that both ANP and BNP are useful to evaluate ADR-induced LV dysfunction[10,11] Troponins are proteins found in cardiac and skeletal muscle, and the troponin complex (subunits I, T, and C) on the thin filament regulates the force and velocity of muscle contractions. Cardiac troponin T (TnT) is a specific and highly sensitive marker of myocardial injury and degeneration[12] and the diagnostic and prognostic value of this marker has been established and extensively reported for acute coronary syndromes[13,14] Furthermore, its significance in DCM[15] and ADR-CM[16–18] has been recently reported.

In the present study, we sought to ascertain whether ANP, BNP, and TnT could be predictors for ADR-CM in the rat model, and to investigate the correlation between the serum concentrations of these biomarkers and the functional alternations associated with ADR-induced myocardial damage.

Methods

Experimental Animals

We obtained 33, 8-week-old, male Wistar rats from Sankyo laboratories (Toyama, Japan) for use in the experiments. ADR-CM was induced by weekly administration of 2 mg/kg of ADR (supplied by Kyowa Hakko Co Ltd) via the tail vein for 8 weeks, following the method of Podesta et al[19] All experimental procedures in the study were approved by the Institutional Animal Care and Use Committee of Kanazawa Medical University, which conformed 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 1996). As a control group,

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Table 1 Natriuretic Peptides, TnT, and %FS of LV in ADR-Injected and Control Groups at 6, 8, 10, and 12 Weeks After the First Administration of ADR

	6 w (n=6)		*8 w (n=6)*		*10 w (n=5)*		*12 w (n=3)*	
	ADR	*Control*	*ADR*	*Control*	*ADR*	*Control*	*ADR*	*Control*
%FS of LV (%)	*58.6±3.6*	*56.1±3.7*	*57.7±6.0*	*63.4±10.1*	*50.9±9.7$*	*66.4±15.9*	*36.8±11.9$*	*67.3±4.2*
ANP (pg/ml)	*99.7±15.5*	*184±59.8*	*103.3±94.4*	*204.8±75*	*140.2±58.1*	*224±73.0*	*136.7±15.3*	*150.3±95.1*
BNP (pg/ml)	*81.5±18.4*	*66.6±16.8*	*67.7±20.4*	*63.4±10.1*	*91.8±24.7$*	*69±17.1*	*173.3±15.3$*	*67.3±4.2*
TnT (ng/ml)	*<0.01*	*<0.01*	*0.02±0.015*	*<0.01*	*0.096±0.059*#*	*<0.01*	*1.093±0.819*#*	*<0.01*

TnT, Troponin T, %FS of LV; Ieft ventricular percent fractional shortening; ADR, doxorubicine (adriamycin); ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; w, weeks.
**p<0.05 vs control group; #p<0.005 vs 6 weeks (baseline); $p<0.005 vs control and baseline.*

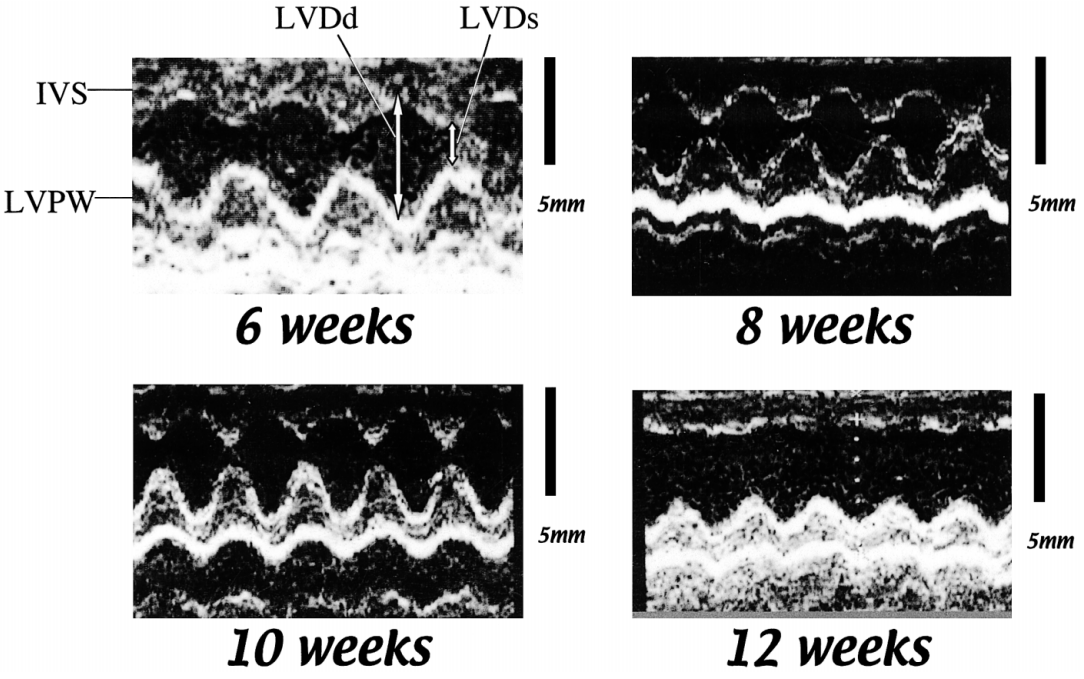

Fig 1. M-mode of left ventricular (LV) echocardiogram at 6, 8, 10, and 12 weeks after the first administration of doxorubicine (ADR). LV contractility diminished at 12 weeks after the first injection of ADR. IVS, intraventricular septum; LVPW, left ventricular posterior wall; LVDd, end-diastolic diameter; LVDs, end-systolic diameter.

physiological saline was injected into rats following the same protocol. Echocardiograms of each rat, anesthetized with ether, were taken at 6, 8, 10 and 12 weeks after the first administration of ADR. Blood samples were collected from the tail vein to quantify plasma ANP, BNP, and serum TnT concentrations after echocardiography, and the hearts of 3 rats were taken out each week.

Analysis of Left Ventricular Performance

LV dimensions [the end-diastolic diameter (LVDd), end-systolic diameter (LVDs), the intraventricular septal thickness, and the LV posterior wall thickness] were measured by echocardiography using a SONOS 5500 with a 12-MHz transducer (Philips, USA). The percent fractional shortening (%FS) of the LV was calculated by the following formula:

$$\%FS = [(LVDd - LVDs)/LVDd] \times 100$$

ANP and BNP Assays

Blood samples were drawn into chilled tubes containing trasylol. Whole blood was centrifuged and the plasma was immediately frozen and stored at –20°C. Radioimmunoassays were performed using commercial kits (Atrial Natriuretic Factor (Rat) S-2039 and Brain Natriuretic Peptide-32 (Rat) S-2078) (Peninsula Laboratories, CA, USA) according to the manufacturer's instructions.

TnT Assay

Blood samples were centrifuged and serum was frozen at –20°C. Serum TnT was measured with a commercial third-generation electrochemiluminescence immunoassay kit (Roche Diagnostics, IN, USA). This method is highly sensitive, compared with those used in previous reports.

Statistical Analysis

All the results are presented as mean ± SD. Statistical significance of differences in ANP and BNP between weeks (6, 8, 10, and 12) and between the ADR and control groups were determined with repeated measure analysis (MIX model in SAS). The statistical significance of differences in %FS and TnT between weeks (6, 8, 10, and 12) and between ADR and control groups were determined by the t test. Significance was considered at values of $p<0.05$.

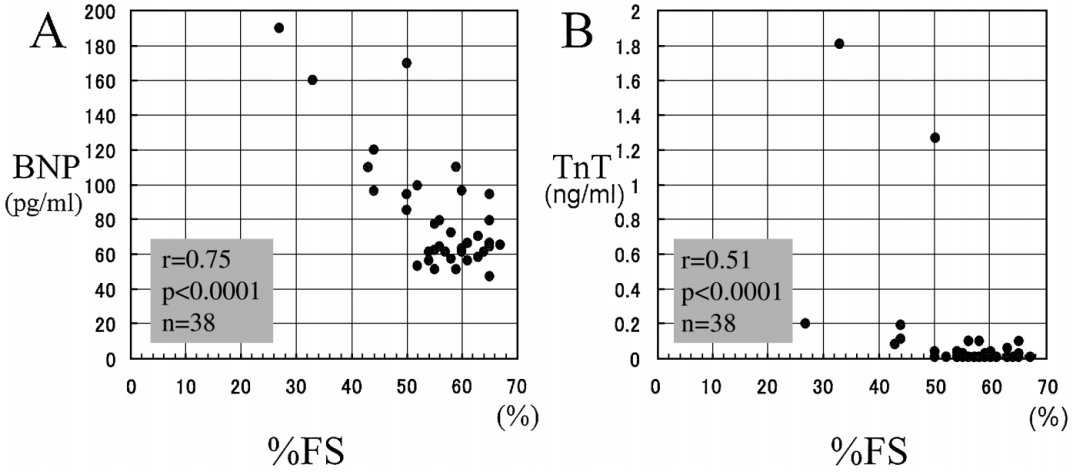

Fig 2. Correlation of percent fractional shortening of the left ventricle (%FS) with concentration of (A) brain natriuretic peptide (BNP) and (B) troponin T (TnT).

Results

LV Performance

The %FS of the LV gradually diminished from 6 to 12 weeks after the first administration of ADR (58.6%, 57.7%, 50.9% and 36.8%, respectively) and had significantly diminished in ADR-injected rats at 10 and 12 weeks after the first injection of ADR compared with the values of control rats (Table 1, Fig 1).

Plasma Concentrations of ANP and BNP

BNP increased from 6 to 12 weeks after the first administration of ADR (81.5, 67.6, 91.8 and 173.3 pg/ml, respectively) and had significantly increased in ADR-injected rats at 10 and 12 weeks after the first injection of ADR, compared with the values of control rats ($p=0.003$ between weeks, $p=0.0035$ between groups, and $p=0.004$ in interaction (week and group) (Table 1). ANP concentrations in ADR-injected rats were not different from those of control rats.

Serum Concentration of TnT

TnT gradually increased from 6 to 12 weeks after the first administration of ADR (<0.01, 0.02, 0.10 and 1.09 ng/ml, respectively); especially significantly ($p<0.01$), it increased from 10 to 12 weeks, at 10 ($p<0.05$) and at 12 ($p<0.01$) weeks after the first administration of ADR, compared with the values of control rats (Table 1). With the method used in the present study, TnT <0.01 ng/ml indicates that the level was not detectable.

Correlation of %FS With BNP and TnT

There were significant negative relationships between BNP and %FS (Fig 2), and between TnT and %FS (Fig 2). TnT in 3 of 6 rats had mildly increased at 8 weeks after the first administration of ADR before the deterioration of %FS. At 10 weeks, TnT in all rats (n=5) had moderately increased with the deterioration of %FS, and at 12 weeks, TnT in all rats (n=3) had markedly increased. BNP in all rats had not increased at 8 weeks after the first administration of ADR and 10 weeks, BNP in 3 of 5 rats had mildly increased with the deterioration of % FS, and at 12 weeks, BNP in all rats (n=3) had moderately increased, similar to TnT. The increase in TnT was antecedent to the increase in BNP in most rats.

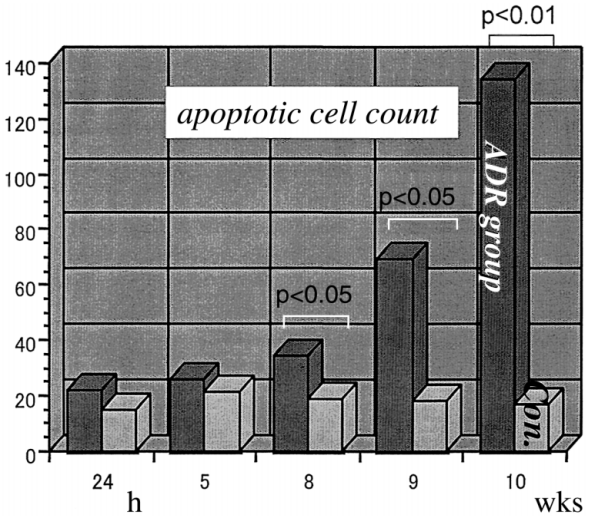

Fig 3. Changes in the number of apoptotic cardiomyocytes in the ADR-injected (ADR group) and control (Con) groups from 24 h to 10 weeks (wks) after the first injection of ADR (Reproduced with permission from Nakamura T et al. Fas-mediated apoptosis in adriamycin-induced cardiomyopathy in rats: In vivo study. *Circulation* 2000; **102:** 572–578).

Discussion

This study showed that BNP and especially TnT were useful as predictors for LV dysfunction caused by ADR-induced myocardial damage in the rat. Although the chronic and late-onset cardiotoxicity of ADR causes irreversible myocardial damage resulting in DCM with fatal congestive failure, ADR still remains a major antitumor agent, so it is clinically important to predict the impairment of LV performance by ADR. LVEF determinations by echocardiography or radionuclide ventriculography are widely used, but their sensitivity and specificity for early detection of cardiotoxicity are limited[3,4] In this study, natriuretic peptides and troponin T were serially and prospectively measured to predict the deterioration of LV dysfunction from ADR-

induced cardiotoxicity, and the relationships between them and %FS of the LV were analyzed.

The diagnostic and prognostic value of TnT for acute coronary syndrome has been established and extensively reported[13,14] and recently, the usefulness of this marker as an early detector of the deterioration of DCM and ADR-CM has also been reported![15–18] Though the mechanisms of myocardial degeneration in DCM are not fully understood, TnT in DCM seems to indicate subclinical myocardial degeneration. Apoptosis, necrosis, calcium handling abnormalities, renin–angiotensin system, endothelin, inflammatory cytokines, nitric oxide, oxidative stress, and mechanical stress have all been invoked as mechanisms[20,21] In previous studies, we demonstrated that apoptotic cell death occurred in the heart of ADR-CM rats, and that the number of apoptotic myocardial cells increased with deterioration of morphological findings and LV function. In the present study, serum concentrations of TnT, which were more specific and sensitive using a third-generation assay, significantly increased from 10 to 12 weeks after the first administration of ADR, the time at which the apoptotic index was increasing (Fig 3) and LV dysfunction was progressive in our earlier study[5] These results suggest that in ADR-CM the increase in the serum concentration of TnT resulted from apoptotic myocardial cell death, although the study of the apoptotic index was not done in the same animals. Further, TnT increased before LV function deteriorated, and so it appears useful to measure the TnT concentration as a predictor LV dysfunction caused by ADR-induced myocardial injury. Previous reports have also demonstrated that TnT is a good monitors of the cardiotoxicity of ADR;[15–18] however, those studies were not prospective or serial, only showing that TnT was an early detector, not a predictor, of deteriorating LV dysfunction.

Plasma levels of natriuretic peptides increase in patients with severe CHF and also in patients with asymptomatic LV dysfunction[22,23] ANP and BNP are also useful makers of LV dysfunction in patients undergoing anthracycline therapy![10,11,24–26] However, ANP is mainly synthesized in the atria and secreted in response to atrial overload, so the mechanism of its release is secondary to any change in ventricular dysfunction, the consequence of which is atrial dilatation. In the present study, ANP did not increase in comparison with the levels of the control rats, which is a different result to that of previous reports![10,11] On the other hand, BNP is synthesized in ventricle and there is much evidence of its usefulness as a detector for heart failure[26] It is thus reasonable that BNP is a more sensitive and specific marker of LV dysfunction than ANP. In the present study, BNP concentrations significantly increased compared with those of control rats at 10 weeks after the first administration of ADR, when marked histopathological changes of cardiomyocytes appeared and LV function deteriorated. These results demonstrate that this prospective study was reliable, and showed that BNP was useful for predicting the progression of ADR-induced myocardial damage. The levels of BNP also had significantly closer correlations with %FS than with TnT ($r=-0.75$ ($p<0.0001$), $r=-0.51$ ($p<0.001$), respectively). However, as the increase in TnT was antecedent to the increase in BNP, TnT seems to be more sensitive than BNP for early detection of LV dysfunction, because a very small increase of TnT was detected by the highly sensitive assay method used in this study.

To prevent anthracycline-induced cardiotoxicity, cardioprotective agents such as dexazoxane;[16] probucol[28] and anti-Fas ligand antibody[6] have been used clinically or experimentally; however, all have limitations. Beta-blockers, especially carvedilol and angiotensin-converting enzyme inhibitors, which have cardioprotective effects, have been shown to be clinically effective for patients with chronic heart failure, including DCM[29] Anti-Fas L antibody showed a partial effect in protecting cardiomyocytes from the apoptosis caused by ADR;[6] however, there is no clinical evidence. Probucol, carvedilol, and angiotensinII AT_2 receptor antagonist have not shown any cardioprotective effect in our ADR-CM rat model (data not shown). Improvements in the procedures to administer those drugs to rats should be considered. Further, our previous study demonstrated quantitatively that a new anthracycline derivative, pirarubicin, had a significantly lower chronic cardiotoxicity in our rat model compared with ADR. That agent has already been used clinically for patients with a variety of malignancies[30] Measurement of both BNP and TnT may also be useful for detecting the effects of these cardioprotective agents, and the degree of cardiotoxicity of the new anthracycline derivative.

In summary, BNP, TnT and %FS of LV were serially and prospectively measured and the results showed that BNP and TnT were useful as predictors for ADR-CM. In particular, TnT, measured by a third-generation assay,[31] was specific and sensitive for the early detection of the deterioration of LV function. Additionally, measurement of BNP and TnT concentrations may be valuable in clinical trials to evaluate the cardiotoxicity of new chemotherapeutic agents, and in experimental and clinical trials assessing the effects of new cardioprotective agents.

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