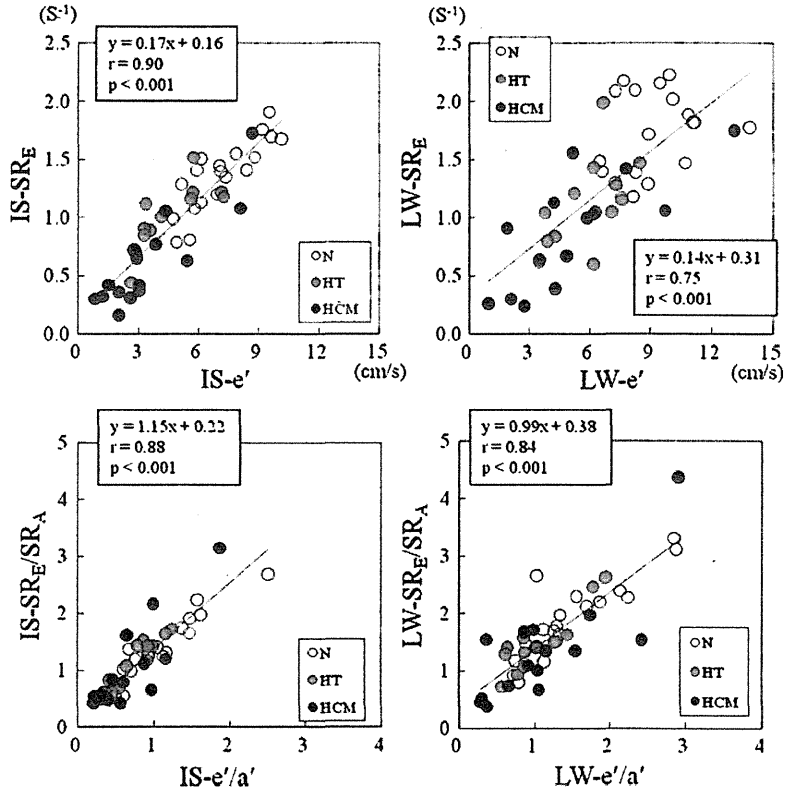

Figure 3 Correlations between diastolic mitral annular velocity and myocardial strain rate of the corresponding wall. e′, peak early diastolic mitral annular velocity; e′/a′, the ratio of the e′ to the peak late diastolic mitral annular velocity; SR_E, peak early diastolic strain rate; SR_E/SR_A, the ratio of SR_E to the peak late diastolic strain rate; HCM, patients with hypertrophic cardiomyopathy; HT, patients with hypertension; IS, interventricular septum; LW, left ventricular lateral wall; N, control subjects.

G-SR_E/SR_A ($r = 0.89$, $P < 0.001$ and $r = 0.90$, $P < 0.001$, respectively). The correlations between LW-e′ and G-SR_E ($r = 0.80$, $P < 0.001$) and between LW-e′/a′ and G-SR_E/ SR_A ($r = 0.82$, $P < 0.001$) were also good but a little weaker.

Relationship between mitral annular velocities and left ventricular global function

Both IS-e′ and M-e′ were well correlated with FPV ($r = 0.77$, $P < 0.001$ and $r = 0.78$, $P < 0.001$, respectively) (*Figure 5*) and significantly correlated with transmitral E ($r = 0.48$, $P < 0.001$ and $r = 0.47$, $P < 0.001$, respectively), DT ($r = 0.49$, $P < 0.001$ and $r = 0.45$, $P < 0.01$, respectively), and IRT ($r = 0.55$, $P < 0.001$, and $r = 0.54$, $P < 0.001$, respectively). IS-e′/a′ and M-e′/a′ were significantly and well correlated with the transmitral E/A ratio ($r = 0.74$, $P < 0.001$ and $r = 0.79$, $P < 0.001$, respectively). LW-e′ was significantly correlated with E ($r = 0.44$, $P = 0.020$), DT ($r = 0.39$, $P < 0.05$), IRT ($r = 0.50$, $P < 0.001$), and FPV ($r = 0.75$, $P < 0.001$), and LW-e′/a′ was also significantly correlated with E/A ($r = 0.75$, $P < 0.001$). However, those correlation coefficients were tended to be slightly weaker.

Discussion

In the present study, IS-e′ was excellently correlated with IS-SR_E, and LW-e′ was fairly well correlated with LW-SR_E, indicating that e′ well reflects LV longitudinal myocardial relaxation. The use of e′ as a parameter of LV diastolic function seems to be based on the assumption that it reflects LV longitudinal myocardial relaxation, but this assumption has not been confirmed. Recently, Opdahl *et al.* reported that a good correlation was observed between early diastolic mitral annular velocity measured using TDI and myocardial relaxation assessed by sonomicrometry in mongrel dogs.[17] However, no sufficient evidence for human has been provided in previous studies. The present study validated the assumption in human and provided better understanding of the property of e′ as an index for LV diastolic function.

In the recommendation for the evaluation of LV diastolic function issued by the American Society of Echocardiography, e′ plays an important role for the management of patients with heart failure.[18] However, there has been no established consensus on the measurement site, that is, whether we should measure e′ at the IS annulus, lateral side annulus, or both. In the present study, the correlation between IS-e′ and IS-SR_E was significantly better than that between LW-e′ and LW-SR_E. In addition, IS-e′ and M-e′

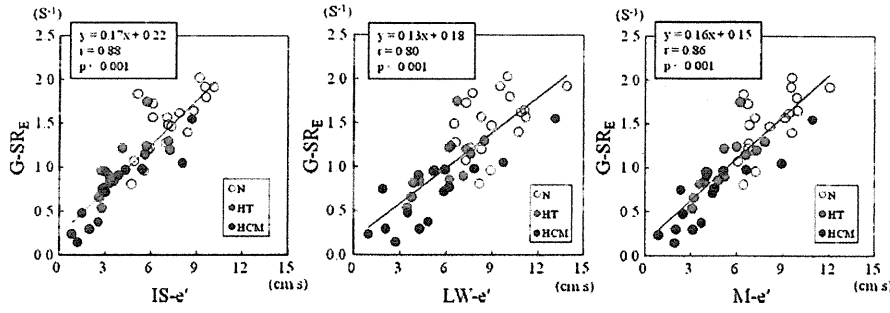

Figure 4 Correlations between the early diastolic myocardial 'global' strain rate ($G\text{-}SR_E$) and early diastolic mitral annular velocities in the interventricular septum (IS), lateral wall (LW), and their mean (*M*). e', peak early diastolic mitral annular velocity; HCM, patients with hypertrophic cardiomyopathy; HT, patients with hypertension; N, control subjects.

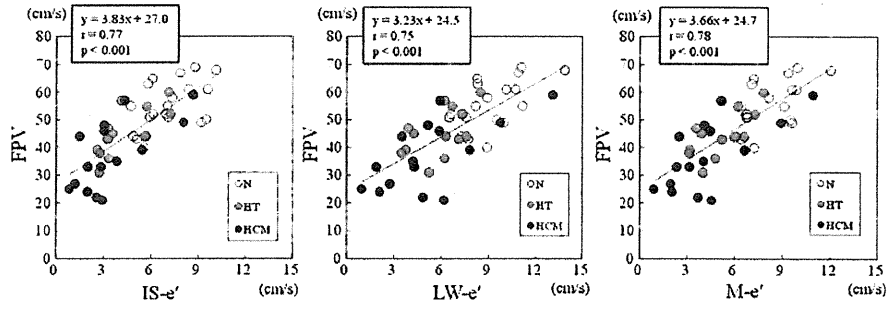

Figure 5 Correlations between left ventricular early diastolic flow propagation velocity (FPV) and early diastolic mitral annular velocities in the interventricular septum (IS), lateral wall (LW), and their mean (*M*). e', peak early diastolic mitral annular velocity; HCM, patients with hypertrophic cardiomyopathy; HT, patients with hypertension; N, control subjects.

were excellently correlated with $G\text{-}SR_E$, and these correlations were slightly better than that between LW-e' and $G\text{-}SR_E$. These results suggest that IS-e' is more useful in assessing LV diastolic function than LW-e'.

There have been several reports comparing IS-e' and LW-e'. Peverill *et al.*[19] reported that LW-e' was greater than IS-e' and the correlation between the two parameters was inadequate. Park *et al.*[20] also indicated a discrepancy between the two measurements and concluded that IS-e' tended to overestimate the LV diastolic dysfunction. However, they did not show any clear evidence to indicate the superiority of LW-e' over IS-e'. In the present study, the correlation between IS-e' and $IS\text{-}SR_E$ was significantly better than that between LW-e' and $LW\text{-}SR_E$. On the other hand, the correlation between LW-e'/*a*' and $LW\text{-}SR_E/SR_A$ was clearly better than that between LW-e' and $LW\text{-}SR_E$. These results indicate that the inadequate correlation between e' and SR_E in the LW is attributable to the angle dependency of TDI. Thus, the IS-e' can more accurately reflect the LV longitudinal myocardial relaxation. In this study, *M*-e' reflected LV diastolic function with a similar accuracy as IS-e'. It is estimated that *M*-e' may be useful in the evaluation of patients with regional myocardial dysfunction such as myocardial ischaemia and regional LV hypertrophy, and in those with right ventricular disease and/or increased right ventricular pressure, which may affect the IS-e' value.

In this study, we employed SR_E measured by using 2DSTI as the standard of longitudinal myocardial relaxation. The accuracy of this method has been reported by several investigators based on comparisons with sonomicrometry and with tagging magnetic resonance imaging.[13,21–23] The e' measured using TDI is widely used as an index for global diastolic function rather than the regional one, based on the hypothesis that mitral annular motion reflects longitudinal shortening and lengthening of the myocardium from the base to the apex. Thus, we measured the so-called 'semi-global' SR_E with ROIs in the whole IS and the whole LW for the comparisons with IS-e' and LW-e', respectively.

Recently, there have been several reports showing the superiority of E/SR_E over *E*/e' in the estimation of the LV filling pressure. Dokainish *et al.*[24] reported that in patients with an LV ejection fraction of $\geq$50% and an *E*/e' value from 8 to 15, E/SR_E by 2DSTI was a better predictor of LV filling pressure than *E*/e' by TDI. In addition, Ng *et al.*[25] reported that E/E_{2D} (E_{2D}: LV myocardial velocity derived from 2DSTI) was more useful in identifying elevated LV end-diastolic pressure than *E*/e'. Although these results should

be sufficiently appreciated, e′ measured using TDI has inevitable merits in clinical practice because it can be very easily measured with good reproducibility and there is a great amount of accumulated clinical evidence supporting its use. In addition, 2DSTI requires high-end ultrasound devices, and its analysis requires good image quality of the whole LV. Furthermore, because the 2DSTI's data analysis algorithm varies according to the ultrasound manufacturer, the criterion value remains unclear. At the present time, 2DSTI is not being administered in all routine examinations.

There are some limitations to be acknowledged in the present study. First, we employed colour TDI instead of pulsed TDI, which is more widely used in the clinical settings. To evaluate the correspondence between TDI and 2DSTI, we thought it better to perform these two measurements offline from the same image over using pulsed TDI, which requires some additional procedures during examination. Because the correspondence in the mitral annular velocity between colour TDI and pulsed TDI was reported to be excellent,[26] the results of this study may be readily applicable to measurements using pulsed TDI. Secondly, e′ and SR_E were studied only in the IS and LW in the four-chamber view. Analyses using more imaging planes might provide a better measuring site of e′ or a better averaged value. However, we consider that the analysis of the four-chamber view is sufficient from a practical view; this plane is very commonly used, and multiplane analysis may limit the ease of this measurement, which is the greatest merit of TDI. Thirdly, the population of this study was too small to perform subgroup analysis. Further study may be needed to evaluate differences among diseases.

Conclusions

In conclusion, early mitral annular velocity (e′), derived using TDI, especially measured at the septal side annulus, well reflects the LV longitudinal myocardial relaxation. The e′ measured at the septal annulus should be used for the clinical evaluation of LV diastolic function rather than the value at the lateral annulus.

Conflict of interest: none declared.

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Hyperuricemia predicts adverse outcomes in patients with heart failure

Sanae Hamaguchi, Tomoo Furumoto, Miyuki Tsuchihashi-Makaya, Kazutomo Goto, Daisuke Goto, Takashi Yokota, Shintaro Kinugawa, Hisashi Yokoshiki, Akira Takeshita [1], Hiroyuki Tsutsui * and for the JCARE-CARD Investigators

Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan

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ABSTRACT

Background: Hyperuricemia is associated with worse outcomes of patients with chronic heart failure (HF). However, it is unknown in an unselected HF patients encountered in routine clinical practice. We thus assessed the impact of hyperuricemia on long-term outcomes including mortality and rehospitalization among patients hospitalized with worsening HF.

Methods: The Japanese Cardiac Registry of Heart Failure in Cardiology (JCARE-CARD) studied prospectively the characteristics and treatments in a broad sample of hospitalized HF patients and the outcomes were followed for 2.1 years after discharge. Study cohorts ($n=1869$) were divided into 2 groups according to serum uric acid (UA) at discharge; ≥7.4 mg/dL ($n=908$) and <7.4 mg/dL ($n=961$).

Results: Of the total cohort of HF patients, 56% had hyperuricemia defined as UA≥7.0 mg/dl. Patients with UA≥7.4 mg/dL had higher rates of all-cause death, cardiac death, rehospitalization, and all-cause death or rehospitalization due to worsening HF. After multivariable adjustment, higher UA levels were a significant and independent predictor for all-cause death (adjusted hazard ratio [HR] 1.413, 95% confidence interval [CI] 1.094–1.824, $P=0.008$) and cardiac death (adjusted HR 1.399, 95% CI 1.020–1.920, $P=0.037$).

Conclusions: Hyperuricemia was common in patients with HF encountered in clinical practice and higher UA was independently associated with long-term adverse outcomes in these patients.

1. Introduction

High serum uric acid (UA) or hyperuricemia has been well demonstrated to be associated with morbidity and mortality in general population [1–3] as well as in patients with coronary artery disease [4,5]. It is also associated with poor outcomes in patients with mild to severe heart failure (HF) [6–9]. Hyperuricemia in HF may be due to the upregulation of the xanthine oxidase (XO), a key enzyme in the generation of oxygen free radicals. Therefore, it may induce proinflammatory activation [10], impaired oxidative metabolism [11], vascular endothelial dysfunction [12], and exercise intolerance [13,14] in HF. These conditions may well explain the association between hyperuricemia and poor outcome in chronic [6,8] as well as acute HF [9]. However, previous studies enrolled small numbers of HF patients ($n=100$–500) and were performed in a single center [6,8,9]. The impact of hyperuricemia on outcomes has not been assessed in a broad cohort of HF patients. Therefore, the purpose of this study was to examine the prevalence of hyperuricemia in HF patients encountered in routine clinical practice and to determine whether it is independently associated with the long-term outcomes. We analyzed the data from the Japanese Cardiac Registry of Heart Failure in Cardiology (JCARE-CARD), a prospective database of the clinical characteristics, treatments, and outcomes in a broad sample of patients hospitalized with worsening HF in Japan [15–19].

2. Materials and methods

2.1. Study patients

The details of the JCARE-CARD have been described previously [15]. Briefly, eligible patients were those hospitalized due to worsening HF as the primary cause of admission. The patients with acute HF were excluded. For each patient, baseline data obtained at discharge included (1) demography; (2) causes of HF; (3) precipitating causes; (4) comorbidities; (5) complications; (6) clinical status; (7) electrocardiographic and echocardiographic findings; (8) plasma brain-type natriuretic peptide (BNP); and (9) treatments including discharge medications. Histories of hypertension, diabetes mellitus, hyperlipidemia, prior stroke, chronic obstructive pulmonary disease (COPD), smoking, prior myocardial infarction, and sustained ventricular tachycardia/fibrillation (VT/VF) were recorded if they were documented at the discharge of index hospitalization. The definition of each comorbidity was described in our previous report [15]. The diagnosis of atrial fibrillation (AF) was based on a 12-lead standard electrocardiogram performed during the hospitalization.

The JCARE-CARD enrolled a total of 2675 patients hospitalized for HF at 164 participating hospitals. Individual participating hospitals entered the data using a web-based electronic data capture (EDC) system licensed by the JACRE-CARD (www.jcare-card.jp). 806 patients were excluded with missing data of serum uric acid, resulting in 1869 patients included in this analysis. They were divided into 2 groups according to serum UA levels at discharge; ≥7.4 mg/dL ($n=908$) and <7.4 mg/dL ($n=961$).

2.2. Outcomes

The status of all patients was surveyed after discharge and the following information was obtained: (1) survival, (2) causes of death, and (3) the rehospitalization due to an

* Corresponding author. Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060-8638, Japan. Tel.: +81 11 706 6970; fax: +81 11 706 7874.
E-mail address: htsutsui@med.hokudai.ac.jp (H. Tsutsui).

[1] Deceased on March 15 2009.

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exacerbation of HF that required more than continuation of their usual therapy on prior admission. Only patients who survived the initial hospitalization were included in the follow-up analysis. Out of 1869 patients, 104 patients (5.6%) who died during the hospitalization and 145 patients (7.7%) who were missed during the follow-up were excluded from the follow-up analysis. Follow-up data were obtained in 1620 out of 1869 patients (86.7%). Mean post-discharge follow-up was 777 ± 312 days (2.1 ± 0.9 years).

2.3. Statistical analysis

Patient characteristics and treatments were compared using Pearson chi-square test for categorical variables and Mann–Whitney *U* test for continuous variables. Multiple linear regression analysis was used to select those variables that were significantly associated with serum UA levels. The model was obtained by using a stepwise regression selection. Cumulative event-free rates during the follow-up were derived using the method of Kaplan and Meier. The relationship between the serum UA level at baseline and outcomes was evaluated among patients with multivariable adjustment. Baseline clinical variables, treatment factors, and the severity of HF at discharge were used in developing the post-discharge Cox proportional hazard models. A *P* value of <0.05 was used for criteria for variables to stay in the model. SPSS version 16.0 J for Windows was used for all statistical analyses.

3. Results

3.1. Patient characteristics

Fig. 1 shows the distribution of serum UA among 1869 patients. Mean serum UA level in the study subjects was 7.3 ± 2.4 mg/dL, ranging from 0.3 to 22.5 mg/dL. 1041 (55.7%) patients had hyperuricemia defined as serum UA ≥ 7.0 mg/dL.

The mean age of the total cohort was 71.1 ± 12.9 years and 60.0% was men (Table 1). The causes of HF were ischemic in 32.5%, valvular in 28.5%, hypertensive in 25.9%, and dilated cardiomyopathy in 17.7%. The mean echocardiographic left ventricular ejection fraction (LVEF) was 44.6 ± 16.4%.

Patients with serum UA ≥ 7.4 mg/dL were more often men and significantly higher body mass index (BMI) (Table 1). Causes of HF did not differ between groups. They were more likely to be smoker and have chronic atrial fibrillation and coronary artery bypass grafting (CABG). Serum creatinine and plasma BNP levels were significantly higher and estimated glomerular filtration rate (eGFR) was lower in patients with serum UA ≥ 7.4 mg/dL. They had greater LV end-diastolic and end-systolic diameters and lower LVEF. The implantations of ICD, CRT, and CRT-D were not significantly different between 2 groups.

Patients with serum UA ≥ 7.4 mg/dL were prescribed more by diuretics, especially loop diuretics, and digitalis at discharge (Table 2). However, the use of other medications such as angiotensin converting enzyme (ACE) inhibitor, angiotensin receptor blocker (ARB), and β-blocker did not differ between groups.

3.2. Variables associated with serum UA levels

In a multiple linear regression analysis, younger age [standardized partial regression coefficients (β) 0.183, $P<0.001$], male gender (β 0.092, $P=0.013$), lower eGFR (β 0.405, $P<0.001$), higher hemoglobin concentration (β 0.120, $P=0.004$), and diuretics use (β 0.103, $P=0.003$) were significantly associated with serum UA levels. Low eGFR was the most important factor in this model. However, the multiple correlation coefficient (R^2) of the model entered these five variables was 0.190, indicating that the contribution of these variables to serum UA levels would be minor.

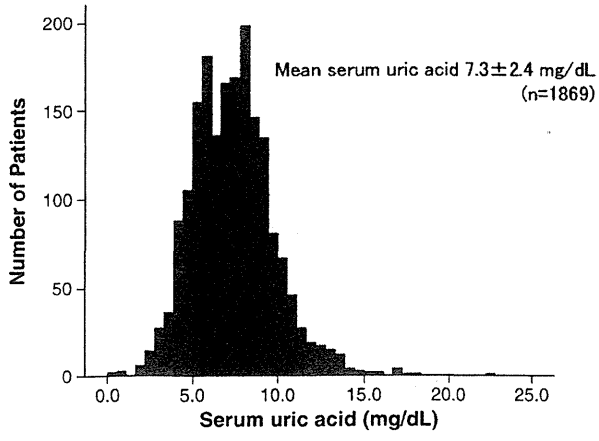

Fig. 1. The distribution of serum UA (mg/dL) at baseline among 1869 patients.

Table 1
Baseline patient characteristics.

Characteristics	Total ($n=1869$)	UA≥7.4 mg/dL ($n=908$)	UA<7.4 mg/dL ($n=961$)	*P* value
Demographics				
Age, yrs (mean ± SD)	71.1 ± 12.9	70.4 ± 14.0	71.8 ± 11.8	0.243
Male, %	60.0	67.4	53.0	<0.001
BMI, kg/m^2	22.3 ± 4.1	22.5 ± 4.1	22.1 ± 4.1	0.013
Causes of heart failure, %				
Ischemic	32.5	33.0	32.0	0.648
Valvular heart disease	28.5	28.7	28.3	0.833
Hypertensive	25.9	24.9	27.0	0.310
Dilated cardiomyopathy	17.7	18.5	17.0	0.383
Hypertrophic cardiomyopathy	1.9	2.1	1.7	0.496
Medical history, %				
Hypertension	53.4	54.4	52.4	0.395
Diabetes mellitus	31.5	32.0	31.1	0.702
Hyperlipidemia	25.0	25.8	24.2	0.436
Prior stroke	16.1	16.7	15.5	0.500
COPD	6.7	7.2	6.2	0.408
Smoking	38.2	43.7	32.9	<0.001
Prior myocardial infarction	27.5	28.2	26.8	0.502
Atrial fibrillation	35.5	38.1	33.0	0.022
Sustained VT/VF	6.5	6.5	6.5	0.968
Previous procedures, %				
PCI	18.5	18.0	19.0	0.590
CABG	9.1	10.6	7.7	0.030
Valvular surgery	7.0	7.1	6.9	0.906
ICD	2.2	2.2	2.2	0.972
CRT	1.6	1.7	1.5	0.830
CRT-D	0.2	0.1	0.2	0.611
Vital signs at discharge				
NYHA functional class	1.8 ± 0.7	1.8 ± 0.7	1.7 ± 0.7	0.006
NYHA classes 3 and 4, %	10.2	11.1	9.3	0.192
Heart rate, bpm	70.6 ± 12.3	70.2 ± 12.4	71.0 ± 12.1	0.156
SBP, mmHg	117.7 ± 19.2	117.1 ± 18.9	118.2 ± 19.4	0.260
DBP, mmHg	66.2 ± 11.9	66.1 ± 12.4	66.3 ± 11.4	0.938
Laboratory data at discharge				
Serum creatinine, mg/dl	1.4 ± 1.2	1.6 ± 1.3	1.2 ± 1.1	<0.001
eGFR, ml/min/1.73 m^2	51.1 ± 25.2	42.4 ± 20.9	58.5 ± 26.1	<0.001
Hemoglobin, g/dL	12.1 ± 2.6	12.0 ± 2.7	12.1 ± 2.6	0.289
Plasma BNP, pg/ml	403 ± 539	485 ± 643	327 ± 405	<0.001
Echocardiographic data at discharge				
LV EDD, mm	55.7 ± 10.3	57.1 ± 10.9	54.4 ± 9.5	<0.001
LV ESD, mm	43.0 ± 12.3	44.8 ± 12.8	41.4 ± 11.6	<0.001
LVEF, %	44.6 ± 16.4	42.8 ± 16.4	46.1 ± 16.3	0.002

BMI, body mass index; COPD, chronic obstructive pulmonary disease; VT/VF, ventricular tachycardia/fibrillation; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; ICD, implantable cardioverter defibrillator; CRT, cardiac resynchronization therapy; CRT-D, cardiac resynchronization therapy device with defibrillator; NYHA, New York Heart Association; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; BNP, brain-type natriuretic peptide; LV, left ventricular; EDD, end-diastolic diameter; ESD, end-systolic diameter; EF, ejection fraction. Values are percent or means ± SD.

3.3. Outcomes

During the follow-up of 2.1 years after hospital discharge, the rates of adverse outcomes were as follows; all-cause death 21.0%, cardiac death 13.5%, rehospitalization due to the worsening HF 36.5%, and all-cause death or rehospitalization 43.9% (Fig. 2). These event rates were significantly higher in patients with serum UA ≥ 7.4 mg/dL.

On multivariate analysis with patients with serum UA < 7.4 mg/dL as the reference, patients with serum UA ≥ 7.4 mg/dL had adverse risk for all-cause death (adjusted hazard ratio [HR] 1.413, 95% confidence interval [CI] 1.094–

Table 2
Medication use at hospital discharge.

	Total ($n=1869$)	UA≥7.4 mg/dL ($n=908$)	UA<7.4 mg/dL ($n=961$)	*P* value
ACE inhibitor, %	36.9	36.1	37.6	0.527
ARB, %	45.8	45.2	46.4	0.617
β blocker, %	48.3	47.9	48.6	0.785
Diuretics, %	88.6	91.4	85.9	<0.001
Loop diuretics, %	80.1	84.4	76.0	<0.001
Thiazide diuretics, %	3.6	4.1	3.2	0.275
Potassium sparing diuretics, %	41.6	41.2	42.0	0.719
Digitalis, %	31.5	34.0	29.2	0.030
Ca channel blocker, %	25.8	27.3	24.4	0.168
Nitrates, %	24.4	24.0	24.8	0.671
Antiarrhythmics, %	16.4	16.9	15.9	0.579
Aspirin, %	47.1	47.9	46.3	0.491
Warfarin, %	40.8	41.8	40.0	0.438
Statin, %	19.8	19.0	20.6	0.406

ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker.

1.824, $P=0.008$) and cardiac death (adjusted HR 1.399, 95% CI 1.020–1.920, $P=0.037$) (Table 3). Therefore, serum UA levels were significantly associated with long-term adverse outcomes including all-cause death and cardiac death even after adjustment for all other covariates including eGFR and the use of diuretics. They were also associated with rehospitalization due to worsening HF (unadjusted HR 1.248, $P=0.040$) and all-cause death or rehospitalization (unadjusted HR 1.322, $P=0.013$), which, however, did not reach statistical significance after multivariable adjustment (adjusted HR 1.025, $P=0.801$ and adjusted HR 1.089, $P=0.304$) (Table 3). CABG was not significantly associated with any endpoints including all-cause death, cardiac death, rehospitalization, and all-cause death or rehospitalization. ICD implantation was significantly associated with rehospitalization (adjusted HR 2.094, 95% CI 1.340–3.273, $P=0.001$) and all-cause death or rehospitalization (adjusted HR 1.844, 95% CI 1.186–2.868, $P=0.007$). CRT implantation was associated with cardiac death (adjusted HR 2.668, 95% CI 1.164–6.114, $P=0.020$), rehospitalization (adjusted HR 2.248, 95% CI 1.327–3.809, $P=0.003$), and all-cause death or rehospitalization (adjusted HR 2.009, 95% CI 1.192–3.386, $P=0.009$). In contrast, valvular surgery was associated with lower rates of all-cause death (adjusted HR 0.466, 95% CI 0.238–0.910, $P=0.025$) and cardiac death (adjusted HR 0.419, 95% CI 0.184–0.951, $P=0.038$). However, the inclusion of these procedures as covariates in the Cox regression model did not change our original results shown in Table 3.

The independent predictors associated with all-cause death among those entered into the Cox proportional hazard analysis were serum UA, BMI, eGFR, plasma BNP, age, and NYHA functional class (Table 4). There was 6.8% increase in all-cause death for each 1 mg/dL increase in serum UA level ($P=0.017$).

4. Discussion

The present study demonstrated that hyperuricemia was seen in 56% of the patients hospitalized with HF. They had higher serum

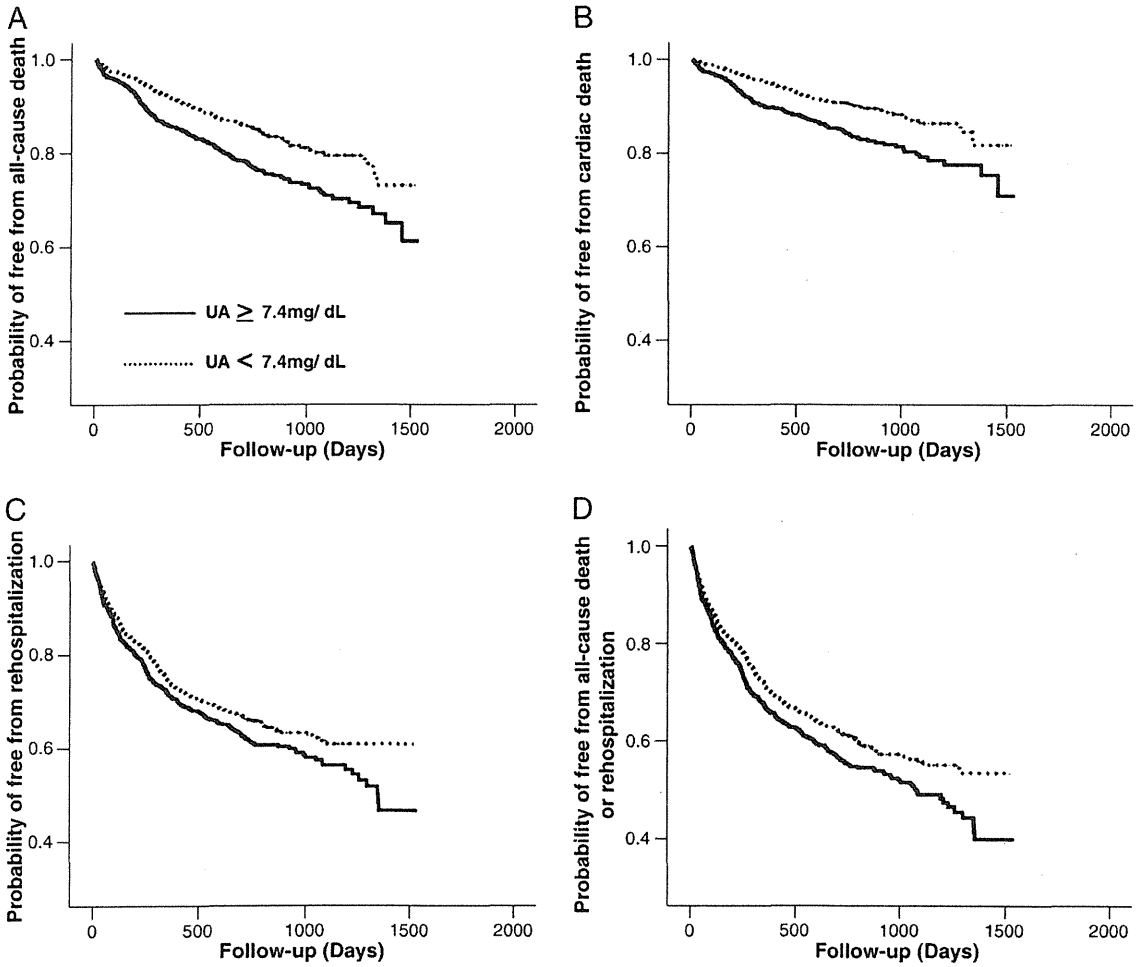

Fig. 2. Kaplan–Meier event-free curves free from all-cause death (A), cardiac death (B), rehospitalization due to worsening HF (C), and all-cause death or rehospitalization (D) comparing patients with serum UA≥7.4 mg/dL (solid lines) and those with serum UA<7.4 mg/dL (dashed lines).

Table 3
Cox analysis for hazard ratios of outcomes associated with the UA level.

Outcomes	Number (%)					
	UA≥7.4 mg/dl (n=776)	UA <7.4 mg/dl (n=844)	Unadjusted HR (95% CI)	P value	Adjusted HR (95% CI)	P value
All-cause death	199 (25.6)	141 (16.7)	1.772 (1.388–2.261)	<0.001	1.413 (1.094–1.824)	0.008
Cardiac death	132 (17.0)	87 (10.3)	1.738 (1.285–2.350)	<0.001	1.399 (1.020–1.920)	0.037
Rehospitalization	303 (39.0)	289 (34.2)	1.248 (1.043–1.494)	0.040	1.025 (0.848–1.239)	0.801
All-cause death or rehospitalization	367 (47.3)	344 (40.8)	1.322 (1.120–1.560)	0.013	1.089 (0.914–1.298)	0.340

The Cox regression model used in the analysis was adjusted for the following covariates: demographics (age, sex, and BMI), medical history (smoking and chronic atrial fibrillation), CABG, NYHA functional class, eGFR, BNP, LVEF, and medication use (diuretics and digitalis). BNP and LVEF at discharge were entered into the model as the categorical variables; i.e. BNP at discharge≥240 pg/ml or <240 pg/ml or unknown and LVEF at discharge<40% or ≥40% or unknown. HR, hazard ratio; CI, confidence interval.

creatinine, higher plasma BNP, and lower LVEF and were prescribed more by loop diuretics and digitalis. Importantly, the risk of adjusted long-term adverse outcomes including all-cause death and cardiac death were significantly higher in patients with UA≥7.4 mg/dl.

Even though the association between UA and cardiovascular diseases, including HF, has remained controversial [20,21], previous studies have demonstrated that UA is an independent risk factor for cardiovascular diseases [2,22,23]. Furthermore, experimental studies have identified mechanisms by which UA induces cardiovascular diseases [24,25]. The present results were consistent with these previous reports [6–9,26,27] and extended their prognostic value to a large, non-selected HF population encountered in routine clinical practice and, more importantly, during the long-term follow-up up to 2.1 years by analyzing the large registry data of hospitalized HF patients.

It should be noted that our results were adjusted with all covariates known to have prognostic value in HF and hyperuricemia was demonstrated to be associated with adverse clinical outcomes independent of renal function and diuretic use (Table 3). In the present study, patients with higher UA had more severe renal dysfunction (Table 1). Renal dysfunction causes hyperuricemia via decreased excretion of UA. Moreover, an elevation of UA level itself can lead to renal dysfunction [25,28–32]. In the present study, the multiple linear regression analysis demonstrated that renal function was the most important factor determining UA level. However, the contribution rate of renal function to serum UA levels was low and serum UA levels were independently associated with the adverse outcomes in HF (Tables 3 and 4). These findings have been also reported by other previous studies [10,11,33]. Therefore, even though serum UA levels can be affected by various factors such as age, gender, renal function, and diuretic use, the present study and other previous studies confirmed that hyperuricemia was independently associated with the adverse clinical outcomes in HF.

The normal UA values are usually higher in men than women. The patients with higher UA levels were more often men in the present study (Table 1). Therefore, the association between UA levels and adverse outcomes might be affected by their gender differences. However, the significant impact of serum UA levels on outcomes was consistently observed even after adjustment with gender (Tables 3 and 4). In addition, to exclude the contribution of gender differences of UA levels, we further analyzed by using the different definition of hyperuricemia based on the genders; >7 mg/dL for men and >6 mg/dL for women. Based on this definition, 1112 (59.5%) patients had hyperuricemia. The prevalence of male was the same between hyperuricemia and no hyperuricemia groups (60.3 vs 59.6%, P=0.770). However, even with the use of different definition of hyperuricemia according to the genders, the relationship between UA and outcomes was consistent with that in our original submission with the UA cut-off values of 7.4 mg/dL.

Table 4
Multivariate predictors of all-cause death by Cox proportional hazard models.

Variables	HR	95% CI	P value
BMI (per 1 kg/m^2 increase)	0.958	0.924–0.993	0.019
eGFR (per 1 ml/min/1.73 m^2 decrease)	1.016	1.010–1.023	<0.001
Serum uric acid (per 1 mg/dL increase)	1.068	1.012–1.127	0.017
Age (per 10 years increase)	1.368	1.214–1.542	<0.001
BNP at discharge ≥240 pg/ml	1.579	1.090–2.287	0.016
NYHA classes 3 and 4 at discharge	1.699	1.165–2.476	0.006

The Cox regression model used in the analysis was adjusted for the following covariates: demographics (age, sex, and BMI), medical history (smoking and chronic atrial fibrillation), CABG, NYHA functional class, eGFR, BNP, LVEF, and medication use (diuretics and digitalis). BNP, LVEF, and NYHA functional class at discharge were entered into the model as the categorical variables; i.e. BNP at discharge≥240 pg/ml or <240 pg/ml or unknown, LVEF at discharge <40% or ≥40% or unknown, and NYHA classes 1 and 2 or 3 and 4. HR, hazard ratio; CI, confidence interval.

There are several mechanisms of hyperuricemia responsible for the increased mortality risk in HF. Serum UA levels may reflect the degree of XO activation in HF [34,35]. XO is one of the major sources of oxygen free radical production and its excess has been shown to be involved in the pathogenesis of HF [36–39]. XO is also shown to impair the regulation of vascular tone [12,33] and reduced vasodilator capacity could lead to exercise intolerance [13,40]. In addition, XO can induce the upregulation of inflammatory cytokines [10]. Hyperuricemia can also reflect an impairment of oxidative metabolism [11]. An inverse relationship between the anaerobic threshold and serum UA concentration has been shown to be present in HF [14]. Finally, hyperuricemia can be a result of renal dysfunction, which may decrease the clearance of UA. However, in the present study as well as other previous studies [11,33], the significant effect of hyperuricemia on outcomes was observed even after the adjustment for risk factors including renal dysfunction.

Several limitations inherent in the design of the registry should be considered. First, the documentation of serum UA levels at hospital discharge might not accurately reflect those after discharge or their changes over time. Second, the information regarding the use of hypouricemiant drugs was not collected in the present study. Similar to the previous studies which also did not collect such information [4–9,27], the critical analysis based on the subgroups with and without the use of hypouricemiant drugs could not be performed. Third, the present study is not a prospective randomized trial and, despite covariate adjustment, other measured and unmeasured factors might have influenced outcomes. For example, serum UA levels have been shown to be higher in patients with postmenopausal state, insulin resistance, elevated leptin level, obstructive sleep apnea, peripheral vascular disease, and movement from rural to urban communities [20]. These factors might be associated with adverse cardiovascular outcomes. Moreover, hyperuricemia is related to inflammation, free radicals and oxidative stress, including XO. However, this study did not collect these data. In addition, the data regarding an indication for surgical treatment were also not collected. However, in the subgroup of prior CABG or valvular surgery higher UA levels were not a significant risk of adverse outcomes either before or after multivariable adjustment. Forth, Cox proportional hazard model has proven to be a useful

approach for identifying the relationships of risk factors. However, such approaches must be interpreted with extreme caution when used to determine the covariates. The other hand, the Cox proportional hazard model for survival analysis has gained widespread use from medical researchers. This is mainly due to the fact that this model is quite well suited for the analysis of epidemiological cohort studies and clinical trials [41]. In fact, this has been used in the previous studies which assessed the relationship between variables including hyperuricemia and survival [8,27]. Fifth, although the present study demonstrated that low BMI values were significant predictors of all-cause death (Table 4), their values themselves were as low as 22 kg/m^2 compared to those in patients from Europe and United States. However, according to the International Study of Macro-Micro nutrients and Blood Pressure (INTERMAP) study [42], the mean BMI values of Japanese middle-aged men and women were 23.7 and 23.2 kg/m^2, respectively, which were much lower than those of 29.1 and 28.7 kg/m^2 in US population, indicating that the low BMI values in our study patients are a population issue of Japanese. Finally, data were dependent on the accuracy of documentation and abstraction by individual medical centers that participated in this study. However, it was not the objective of this study to restrict enrollment to the narrowly defined population of HF usually included in clinical trials but rather to include a broad range of patients reflecting the current reality of clinical practice. Even though we made an extensive effort to better address and focus the limitation of this study, some major limitation may be still present.

In conclusion, the present study demonstrated that hyperuricemia was common in patients hospitalized with worsening HF and independently associated with long-term adverse outcomes in these patients. Further studies are definitely needed to establish the role of serum UA levels as a potential biomarker for the future risk stratification and a therapeutic target for HF.

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Original article

Activation of adenosine A1 receptor attenuates tumor necrosis factor-α induced hypertrophy of cardiomyocytes

Yulin Liao [a,*], Li Lin [a,b], Di Lu [a,b], Yujun Fu [a,b], Jianping Bin [a], Dingli Xu [a], Masafumi Kitakaze [a,c]

[a] Department of Cardiology, Nanfang Hospital, Southern Medical University, 1838, Guangzhou avenue north, Guangzhou, 510515, China
[b] Department of Pathophysiology, China-Japan Collaborative Laboratory of Cardiovascular Physiology and Key Laboratory of Shock and Microcirculation Research, Southern Medical University, Guangzhou 510515, China
[c] Cardiovascular Division of the Department of Medicine, National Cerebral and Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan

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Abbreviations:
ADAM-17, a disintegrin and metalloproteinase-17
CGS21680, 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamino adenosine hydrochloride
CPA, N6-cyclopentyladenosine
IB-MECA, N6-(3-iodobenzyl)-5'-N-methylcarbamoyladenosine
NECA, 5-ethylcarboxamidoadenosine
TAC, transverse aortic constriction
TNF-α, tumor necrosis factor-α

ABSTRACT

Tumor necrosis factor (TNF)-α has been implicated in the pathogenesis of cardiac hypertrophy, while the activation of adenosine receptors has been shown to exert antihypertrophic effect on the heart. However, it remains unknown whether adenosine can attenuate hypertrophy induced by TNF-α. This study was aimed to address this issue using transverse aortic constriction (TAC) mouse models and cultured neonatal rat cardiomyocytes. Plasma TNF-α was significantly increased in hypertrophied hearts (Sham vs TAC group: 46.8 ± 2.5 vs 67.0 ± 1.6 pg/ml, $P = 0.021$), while myocardial TNF-α level, expression of TNF receptor 1 and TNF-α-converting enzyme were positively correlated with heart weight to body weight ratio ($r = 0.930$, 0.676 and 0.891, respectively, $P < 0.01$–0.05). Myocardial adenosine levels were increased significantly at 4 weeks (Sham vs TAC group: 16.15 ± 1.59 vs 86.54 ± 13.49 nmol/mg protein, $P < 0.01$) and decreased from 6 to 11 weeks after TAC. N6-cyclopentyladenosine, an adenosine A1 receptor agonist inhibited protein synthesis of cardiomyocytes induced by TNF-α in a dose-dependent manner. This antihypertrophic effect could not be mimicked by agonists of A2a, A2b and A3 adenosine receptors. These findings indicate that TNF-α signal system plays important role in the process of cardiac hypertrophy, and activation of adenosine receptor 1 inhibits hypertrophy of cardiomyocytes induced by TNF-α.

1. Introduction

It is well known that tumor necrosis factor-α (TNF-α), a ubiquitous cytokine, plays significant roles in various cardiac diseases [1–5]. Emerging evidence demonstrates that TNF-α is associated with myocardial hypertrophy [6–9], one of the pathologic features during the development and progression of heart failure. Recent evidence shows that both TNF-α-converting enzyme (also called "a disintegrin and metalloproteinase", ADAM-17) [6] and soluble tumor necrosis factor receptor 1(TNFR1) [7] have been implicated in the pathogenesis of cardiac hypertrophy. However, few studies have focused on the treatment of hypertrophy induced by TNF-α.

Adenosine is known for its various cardiac beneficial effects by counteracting against adrenergic system [10,11] and rennin-angiotensin-aldosterone system [12], increasing tolerance to hypoxia [13,14], as well as inhibiting fibroblast proliferation and collagen synthesis [15,16]. All these beneficial effects lead to the protection of heart and limit its remodeling and progression to failure. Adenosine transmits its signal through four subtypes of G-protein coupled adenosine receptors (A1, A2a, A2b and A3). These receptors mediate various responses, including modulation of coronary flow, heart rate, myocardial contraction, cardioprotection, inflammation, and cardiac remodeling [17].

In earlier clinical studies, adenosine was shown to increase in patients with chronic heart failure [18] and to attenuate the severity of the disease [19]. Since adenosine signalling plays significant roles in the pathogenesis of a variety of cardiovascular disorders, and it is therefore an attractive system for therapeutic manipulation, and the interests on adenosine still continues. Studies have shown that the endogenous TNF-α production was

* Corresponding author. Tel.: +86 20 62786265; fax: +86 20 87277521.
E-mail address: Liao18@msn.com (Y. Liao).

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inhibited by stimulating cardiac adenosine receptors in the gene transcription level [20–22], implying an anti-TNF-α effect of adenosine. However, to our best knowledge, seldom researches have involved the adenosine function on TNF-α induced hypertrophy of cardiomyocytes.

In this study, we created transverse aortic constriction (TAC) mouse models to induce hypertrophy. Plasma and cardiac TNF-α level and cardiac adenosine level were evaluated to confirm correlation of TNF-α, adenosine and cardiac hypertrophy. Correspondingly, in cellular level, we cultured neonatal rat cardiomyocytes to identify an antihypertrophic role of adenosine and to clarify which type of adenosine receptor activation is responsible for attenuating the pro-hypertrophic effect of TNF-α.

2. Materials and methods

2.1. Agents

Tumor necrosis factor-α (TNF-α), N6-cyclopentyladenosine (CPA), 5-ethylcarboxamidoadenosine (NECA), 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamino adenosine hydrochloride (CGS21680), and N6-(3-iodobenzyl)-5'-N-methylcarbamoyladenosine (IB-MECA) were purchased from Sigma Chemical Company.

2.2. Animal models

All procedures were performed in accordance with our institutional guidelines for animal research and complied with the National Institutes of Health (NIH) Guide. Mice (C57BL/6, male, 7 weeks old, weighing 18 to 25 g) were intraperitoneally anesthetized with a mixture of xylazine (5 mg/kg) and ketamine (100 mg/kg), and transverse aortic constriction (TAC) was induced in the mice by using the methods described in previous studies [23,24].

The C57 BL/6 mice were divided into two groups: sham ($n = 9$) and TAC ($n = 25$); these mice were sacrificed by overdose of pentobarbital (150 mg/kg) and cervical dissociation at 1–11 weeks after the operation to obtain samples showing different degree of cardiac hypertrophy and pulmonary congestion. Blood from the right ventricle was obtained.

2.3. Measurement of plasma and myocardial TNF-α level

Both plasma and homogenated myocardial TNF-α levels were measured by using an ELISA kit (Quantikine, Catalog No. MTA00; R&D SYSTEMS, Minneapolis, USA) according to the manufacturer's instructions.

2.4. Real-time PCR

Total RNA was extracted from homogenized myocardial tissues using Trizol (Invitrogen, USA). Real-time PCR for the mRNAs of TNFR1, ADAM-17 and GAPDH was performed with the ABI Prism 7300 Sequence Detection System (Applied Biosystems Inc. USA) and SYBR Green PCR Master Mix (Toyobo, Japan).

2.5. Cell culture

Neonatal rat ventricular myocytes were isolated as described [25]. Cardiac myocytes were cultured in Dulbecco's Modified Eagle Media (Sigma) supplemented with 10% FBS (Equitech-Bio Inc). Culture media were changed to serum-free at 72 hours. Cardiomyocytes were cultured in serum-free conditions for 48 hours before experiments. Protein synthesis in cultured cells was evaluated by analysis of [^{3}H] leucine incorporation as described elsewhere [26].

2.6. Measurement of myocardial adenosine level

Myocardial adenosine levels were measured by radioimmunoassay after homogenized as previously reported [27].

2.7. Cardiomyocyte hypertrophy assay

Cardiomyocytes were exposed to TNF-α 10 ng/ml for 24 hours in the presence or absence of CPA, and the extent of increase in [^{3}H] leucine uptake was examined [26]. We also studied the effects of A2a (CGS21680), and A3 (IB-MECA) receptor selective agonists and the nonselective agonist (NECA, mainly for A2b) on TNF-α induced cardiomyocyte hypertrophy.

2.8. Statistical analysis

For statistical analyses, comparison between two groups was carried out by t test, while multiple comparisons were performed by 1-way analysis of variance (ANOVA) using Tukey–Kramer exact probability test. The least-squares method was used to assess linear correlation between selected variables. The results were reported as mean ± standard error of mean, and P values of < 0.05 were considered to be statistically significant.

3. Results

3.1. Association between TNF-α signal system and cardiac hypertrophy

To confirm the cardiac remodeling induced by surgery, mice heart and lung were weighted in both TAC and sham group 4 weeks after operation. The operated heart presented different hypertrophy degrees, ranging from none, mild, moderate and sever hypertrophy (Fig. 1A). The plasma TNF-α concentration in TAC group 4 weeks after surgery was elevated in comparison with sham group (Fig. 1B, $P < 0.05$). Myocardial TNF-α level was positively correlated with the heart weight/body weight ratios (HW/BW) ($r = 0.930$, $P < 0.01$; Fig. 1C). Similarly, mRNA expression of TNFR1 and ADAM-17 was also significantly correlated with HW/BW ($r = 0.676$ and 0.891, respectively; Fig. 1D, E).

Above findings indicate that endogenous TNF-α production, expression of TNFR1 and ADMA-17 are closely associated with the development of cardiac hypertrophy. We next examine the time course change of myocardial adenosine during the progression of cardiac remodeling.

3.2. Cardiac adenosine level changes during the process of cardiac hypertrophy development

As shown on Fig. 2, with the progression of cardiac hypertrophy, the cardiac adenosine level fluctuated in TAC models, while the cardiac adenosine level in sham group did not show much change. Myocardial adenosine level in TAC group rose to the peak at 4 week and was about four folds of the sham group ($P < 0.01$). However, at 6 weeks later, the cardiac adenosine level dropped dramatically but still remained a tendency of higher than the sham group. These findings suggest that endogenous adenosine is involved in the process of cardiac remodeling.

3.3. Activating adenosine receptor 1 inhibits TNF-α induced hypertrophy in cardiomyocyte

First, we confirmed repeatedly that stimulation with TNF-α 10 ng/mL increased protein synthesis of cardiomyocytes by about 40% (Fig. 3). In order to verify whether activating adenosine receptors can attenuate pro-hypertrophy effect of TNF-α, we then used agonists of various adenosine receptors. The range safety of

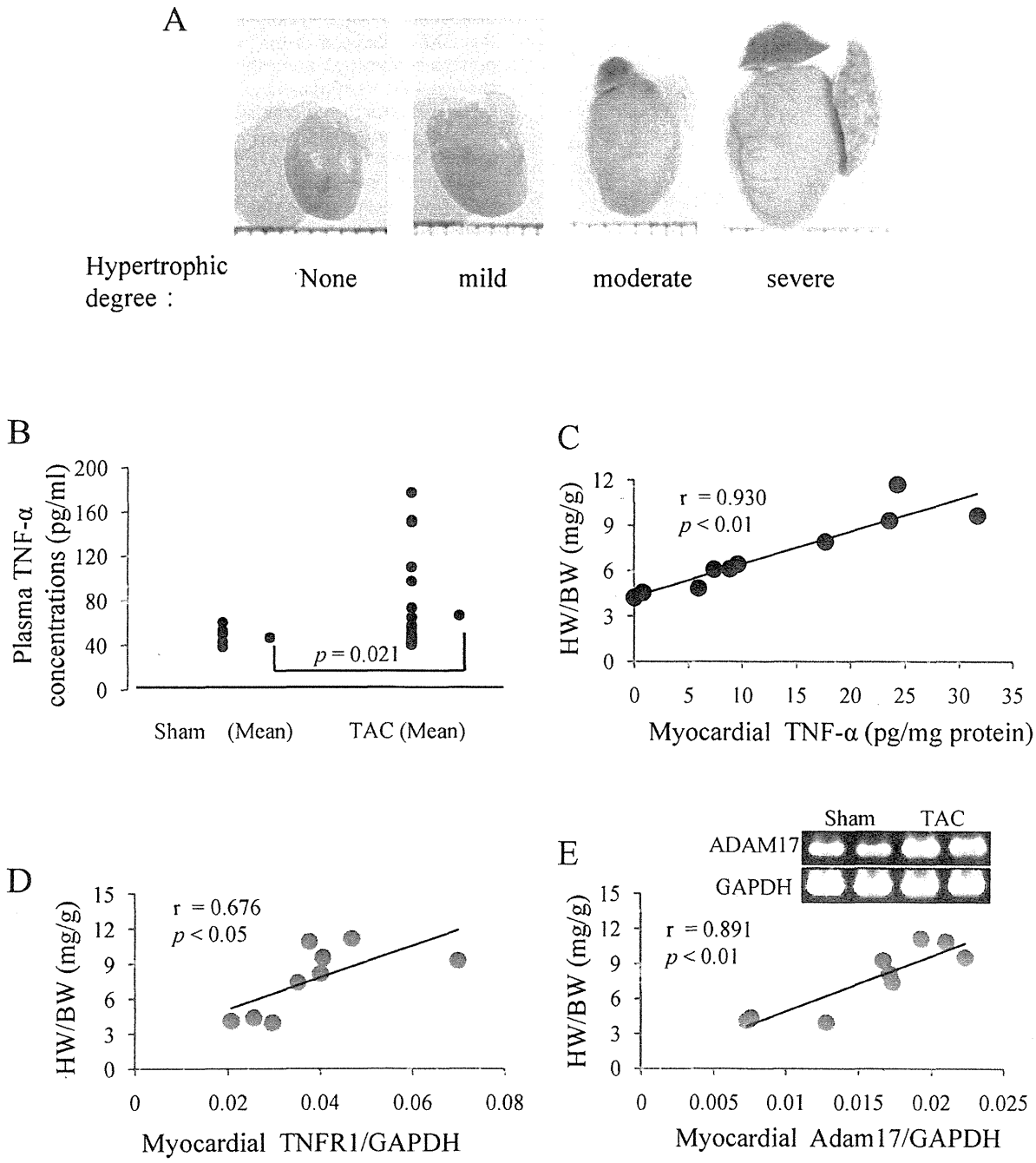

Fig. 1. Correlations between TNF-α signal molecules and cardiac hypertrophy. A. Representative pictures of whole hearts with various degree of hypertrophy. B. At 4 weeks after surgery, TNF-α concentrations were significantly higher in the TAC group ($n = 25$) than in sham group ($n = 9$). C. Correlation between myocardial TNF-α and heart weight/body weight ratio (HW/BW). D. Correlation between myocardial TNFR1 mRNA expression level and HW/BW. E. Correlation between myocardial ADAM-17 mRNA expression level and HW/BW. Values are presented as mean ± SEM or raw data in B.

drug concentrations in cardiomyocytes was identified when treatment with those drugs alone did not significantly reduce the basal [^{3}H] leucine uptake. For CPA, CGS21680, NECA and IB-MECA, the safe concentrations were not higher than 10 μM, 1 μM, 100 μM and 0.01 μM, respectively (Fig. 3A–D).

In the safety range of concentrations, CPA, an agonist of A1 receptor, inhibited TNF-α-induced cardiomyocytes hypertrophy in a concentration-dependent fashion (Fig. 3A), while CGS21680 (an A2a receptor agonist), NECA (a non-selective agonist with relative high selectivity for A2b) and IB-MECA (an A3 selective receptor agonist) did not significantly affected the TNF-α-induced protein synthesis in cardiomyocytes (Fig. 3B–D).

Taken together, CPA abrogated TNF-α-induced hypertrophy, which couldn't be mimicked by A2a, A2b and A3 adenosine receptor agonists. Therefore, we conclude that it is A1, not A2a, A2b or A3 receptors that mediate the antihypertrophic effect.

4. Discussion

TNF-α produced by macrophages or cardiomyocytes participates in the process of hypertrophy [1–4]. Direct inhibition of TNF-α using TNF-α neutralizing antibody was once adopted in clinical trials to treat patients with heart failure but the result was

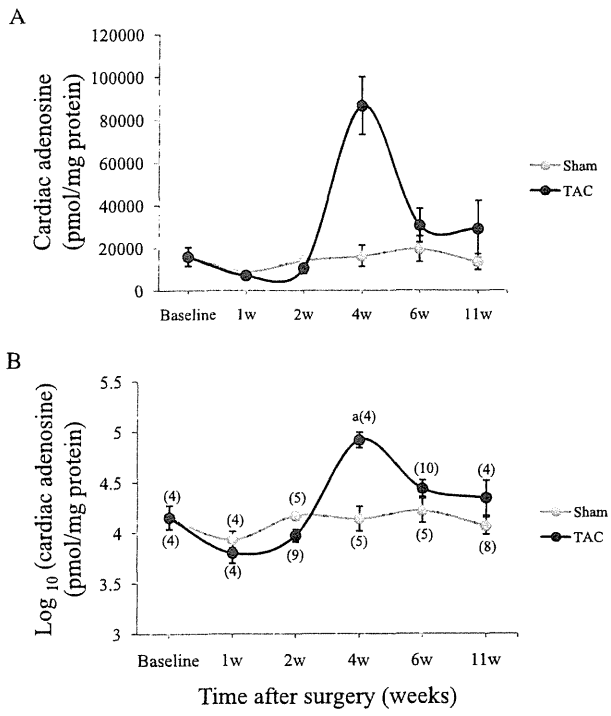

Fig. 2. Temporally changes of myocardial adenosine during cardiac hypertrophy development. Data in the panel A were transformed to logarithmic data as shown in the panel B. In TAC models, the cardiac adenosine level had a transient increase at 4 week after surgery ($^{a}P < 0.01$ vs the corresponding sham group). Values are presented as mean ± SEM. Number of mice is indicated in brackets.

disappointed [28], one explanation is the different role of TNFRs that TNF-α induced cardiac toxicity by binding to TNFR1 and protective effect by binding to TNFR2 [29]. In the present study, we showed that development of cardiac hypertrophy was closely associated with the up-regulation of myocardial TNFR1, ADAM-17 and TNF-α, indicating an important role of TNF-α signal system in cardiac hypertrophy. We further demonstrated that activating adenosine A1 receptors attenuates the pro-hypertrophic effect of the TNF-α in cardiomyocytes, implying a new strategy for TNF-α inhibition. In our previous study, we have demonstrated that activation of adenosine A1 receptors inhibits protein synthesis of neonatal rat cardiomyocytes induced by G-protein coupled receptor agonists, and noted that adenosine A1 receptor agonist attenuated cardiac hypertrophy and prevented heart failure in mice with left ventricular pressure overload [23]. Although accumulated evidence has showed adenosine's antihypertrophic effect [23,30] and TNF-α's prohypertropic effect [1,3,4], to our best knowledge, this study is the first showing that pro-hypertrophic effect of TNF-α was blunted by adenosine receptor activation.

In agreement with previous experimental [10,14] and clinical studies [18], we found that myocardial adenosine level was increased initially and then decreased. Cardiac hypertrophy is not a necessary compensatory response since inhibiting cardiac hypertrophy does not worsen but improve heart failure [23,31,32]. Similar to ANP or BNP, we postulate that adenosine level elevation during cardiac hypertrophy may be a compensatory response. Previous clinical observations demonstrated that plasma adenosine levels increased in patients with mild to moderate severity of chronic heart failure [18,19], but it was decreased when heart failure progressed to NYHA class IV, consistent with our findings [18]. A possible explanation may be obtained from the energy metabolism. In the pressure overload mice, cardiomyocytes demand more energy consumption for compensation, and endogenous adenosine would facilitate glucose uptake and improve energy utilization [17]. Therefore, we presume that the

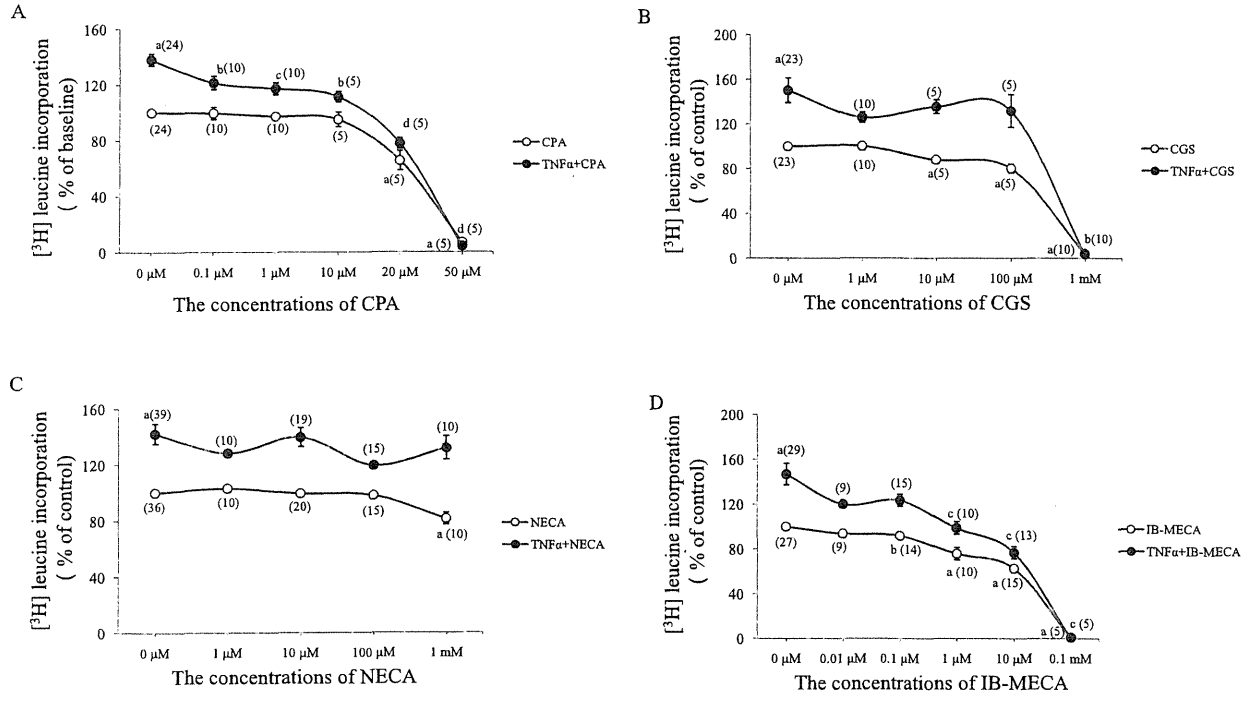

Fig. 3. Effect of adenosine agonists on TNF-α induced protein synthesis of cardiomyocytes. Incorporation of [^{3}H] leucine without addition of any agents (adenosine agonist 0 μM) was served as baseline. A. Safety range of CPA < 20 μM. $^{a}P < 0.001$ vs baseline; $^{b}P < 0.05$, $^{c}P < 0.01$ and $^{d}P < 0.010$ vs TNF-α + CPA 0 μM. B. Safety range of CGS < 10 μM. $^{a}P < 0.001$ vs baseline; $^{b}P < 0.001$ vs TNF-α + CGS 0 μM. C. Safety range of NECA < 1 mM. $^{a}P < 0.001$ vs baseline. D. Safety range of IB-MECA < 0.1 μM. $^{a}P < 0.001$, $^{c}P < 0.001$ vs TNF-α + IB-MECA 0 μM. Number of sample (wells) is indicated in brackets.

fluctuant change of adenosine level is due to the process from compensatory to decompensatory phase of heart failure.

In this study, we used different adenosine analogs to stimulate its receptors. As shown in the study, CPA ameliorated the pro-hypertrophy effect of TNF-α significantly, but it cannot be mimicked by other agonists (CGS for A2a; NECA mainly for A2b; IB-MECA for A3). Accordingly, we posit that the function of anti-TNF-α's pro-hypertrophy effect is initiated by stimulating adenosine A1 receptor, and exclude the effect of other receptors stimulation. Coincidently, it was reported that adenosine reduced the TNF-α expression in cardiomyocytes [20] and cardiac tissue [21].

In conclusion, the data in this study indicate that myocardial TNF-α, TNFR1 as well as ADAM-17 is positively correlated with the degree of cardiac hypertrophy and that the pro-hypertrophic effect of TNF-α is abrogated by the activation of adenosine A1 receptor in cardiomyocytes. However, the influence of adenosine on downstream signal pathway of TNF-α is not involved in this study, and need further exploration.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Original article

Dynamic changes in plasma total and high molecular weight adiponectin levels in acute heart failure

Takahiro Ohara (MD, PhD)[a,*], Kazuhiko Hashimura (MD)[b], Masanori Asakura (MD, PhD)[a], Akiko Ogai (MS)[a], Makoto Amaki (MD, PhD)[a], Takuya Hasegawa (MD)[a], Hideaki Kanzaki (MD, FJCC)[a], Mina Sonoda (MS)[c], Hitoshi Nishizawa (MD, PhD)[c], Tohru Funahashi (MD, PhD)[c], Masafumi Kitakaze (MD, PhD, FJCC)[a]

[a] *Cardiovascular Division, National Cerebral and Cardiovascular Center, 5-7-1 Fujishirodai, Suita 565-8565, Japan*
[b] *Yao Municipal Hospital, Yao, Japan*
[c] *Department of Internal Medicine and Molecular Science, Osaka University Graduate School of Medicine, Suita, Japan*

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KEYWORDS
Cytokines;
Heart failure;
Natriuretic peptide;
B-type

Summary
Background: Elevated levels of total plasma adiponectin (APN) and high molecular weight (HMW)-APN have been observed in chronic heart failure (HF) and are associated with poor prognosis, however, the response of APN levels in acute HF is not known. The purpose of this study was to clarify the dynamic changes of the plasma total APN, HMW-APN levels, and the ratio of HMW-APN to total APN (HMWR) in acute HF.
Methods: From February 2006 to January 2007, 20 patients with acute HF (non-ischemic and non-valvular origin, 17 men, aged 63 ± 11 years) were enrolled, and blood was sampled before the onset of the treatment and at discharge. Ten patients admitted for the treatment of supraventricular arrhythmia (8 men, aged 45 ± 13 years) were included as controls.
Results: The medians and interquartile ranges of the plasma total APN, HMW-APN levels, and HMWR at admission were 20.8 (14.5–38.9) μg/mL, 12.4 (7.7–23.3) μg/mL, and 0.60 (0.50–0.69), respectively. The total APN and HMW-APN values were significantly higher than the values of the control. The plasma total APN, HMW-APN, and HMWR values at discharge decreased to 19.4 (7.2–27.3) μg/mL, 10.5 (3.2–12.8) μg/mL, and 0.52 (0.46–0.57), respectively. An exploratory survival analysis showed that the higher HMWR values at admission and the larger decrease in HMWR were associated with a better prognosis after discharge.
Conclusion: Plasma total APN and HMW-APN values are elevated at the admission for acute HF. Plasma total APN, HMW-APN, and HMWR values decrease following treatment. Higher HMWR at admission and its larger decrease may be the signs of favorable treatment responsiveness in acute HF.

* Corresponding author. Tel.: +81 6 6833 5012; fax: +81 6 6872 7486.
E-mail address: tkohara529@gmail.com (T. Ohara).

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Introduction

Adiponectin (APN) is one of the circulating adipocytokines mainly produced by adipose tissue, and plays an important role in regulating insulin sensitivity, lipid metabolism, and systemic inflammation [1,2]. Circulating APN exists in blood in several multimeric forms. Recent studies have suggested that high molecular weight APN (HMW-APN) is the active form of this hormone and that the HMW-APN level or the ratio of HMW-APN to total APN (HMWR) are the superior predictors of metabolic abnormalities compared with total APN [3].

Heart failure (HF) is a complex syndrome with a high mortality that involves hemodynamic, neurohormonal, and metabolic abnormalities [4]. Recent studies have demonstrated that plasma total APN and HMW-APN levels increased in patients with chronic HF, and the elevated plasma APN levels identified patients with increased risk of dying, although the underlying mechanisms have remained unclear [5–7]. Since APN is believed to be beneficial for both hypertrophic and failing hearts [8,9], the elevated plasma APN levels may be a compensatory response to HF. However, how plasma total APN, HMW-APN, and HMWR respond to acute HF and after its treatment are not known.

Therefore, we designed a prospective observational study to investigate the changes in plasma total APN, HMW-APN levels, and HMWR during the course of acute HF, along with the hemodynamic, neurohormonal, and metabolic parameters and the clinical outcome.

Methods

The study was conducted in accordance with the Declaration of Helsinki, and was approved by the ethics committee of the National Cerebral and Cardiovascular Center. Written informed consent was obtained from each subject before participation in this study.

Subjects

From February 2006 to July 2007, 20 consecutive patients admitted with acute non-ischemic and non-valvular HF were enrolled. Blood was sampled before the onset of any treatment and at discharge. The median and interquartile range of the time interval between the two blood sampling was 45 (21–70) days. The attending physicians treated the patients according to the guidelines for the management of acute HF issued by either the Japanese Circulation Society [10] or the European Society of Cardiology [11]. Ten patients admitted for the treatment of supraventricular arrhythmia without organic heart disease (8 men, aged 45 ± 13 years) were included as the control.

Assays

The plasma concentration of total APN was measured with a specific immunoradiometric assay [12]. Plasma HMW-APN levels were measured with a sandwich enzyme-linked immunoassay (ELISA) that employed a monoclonal antibody for human HMW-APN (Fujirebio ELISA kit, Tokyo, Japan) [13].

The plasma B-type natriuretic peptide (BNP) level was measured with a specific immunoradiometric assay kit for human BNP measurements (Shionoria; Shionogi Co., Ltd., Osaka, Japan) [14]. The insulin resistance index was determined by the homeostasis model assessment of insulin resistance (HOMA-R) method and calculated as the product of the fasting glucose (mg/dL) and fasting plasma insulin level (μU/mL) divided by 405 [15].

Echocardiography

Most of the echocardiograms were performed at the same day of the blood sampling but some of them were performed when the clinical status was stabilized. The median and interquartile range of the interval between the echocardiogram and the blood sampling was 0 (0–6) days. The left atrial diameter, left ventricular end-diastolic and end-systolic dimensions, left ventricular fractional shortening, and left ventricular septal thickness, and posterior wall thickness were determined by M-mode echocardiography. Echocardiograms were read by physicians who were blinded to any clinical information about the subjects. The left ventricular mass was calculated with the parameters measured by M-mode echocardiography [16]. Indexed values were obtained by dividing each parameter by the body surface area which was calculated according to the method of Mosteller [17]. Trans-mitral inflow profile was assessed in the apical four-chamber view by pulsed-wave Doppler echocardiography, with the Doppler beam parallel to the direction of the flow and the sample volume set at the tips of the leaflets. The peak velocity of early diastolic transmitral flow, the peak velocity of late diastolic transmitral flow, and the early diastolic transmitral flow deceleration time were measured [18]. Pulse wave tissue Doppler imaging was performed in the apical four-chamber view to acquire early diastolic mitral annular velocities [18]. The mean value of the septal and lateral values was used.

Follow up

After discharge from the hospital, the occurrence of clinical events was assessed by chart review or telephone interview. All cause death and the admission for worsening of HF were determined as clinical events.

Statistics

Numerical data are reported as either the mean $\pm$ SD or the median and interquartile range, as appropriate. The numerical values at admission and at discharge were compared by the paired t-test or Wilcoxon's matched pairs signed rank test, as appropriate. The categorical values at admission and at discharge were compared by McNemar test. For comparing the values at admission or at discharge with the values of control, Mann–Whitney test was performed. To avoid a type I error, a probability (p) value < 0.025 was considered as significant in this analysis. In the other all analyses, $p < 0.05$ was accepted as indicating statistical significance. The predictors of the plasma total APN and HMW-APN values at admission and at discharge were analyzed with simple

linear regression analysis using the selected variables known to relate with APN values in the previous reports. Variables are log-transformed as appropriate. For an exploratory survival analysis, patients were divided into two groups based on the median values of total APN, HMW-APN, and HMWR at admission and discharge and their changes. Then Kaplan–Meier survival curves were constructed for each of the subgroups and compared by log-rank test. The SPSS software package (16.0.2J; SPSS, Chicago, IL, USA) was used for statistical analysis.

Results

Patient background

The baseline clinical, biochemical, and echocardiographic data are summarized in Table 1. None of the patients took either pioglitazone or fibrates, which can alter the plasma APN levels. Our patients had mostly dilated left ventricle and reduced systolic function. The results of transmitral flow showed that they had mostly severely compromised diastolic function. The majority of the patients were treated with diuretics or vasodilators. A minority of the patients needed positive inotropic agents. None of the patients received non-invasive positive pressure ventilation or endotracheal intubation.

Changes in plasma total APN, HMW-APN, and HMWR values and other variables

The plasma total APN, HMW-APN, and HMWR values at admission were 20.8 (14.5–38.9) μg/mL, 12.4 (7.7–23.3) μg/mL, and 0.60 (0.50–0.69), respectively (Table 2). The plasma total APN and HMW-APN values at admission were significantly higher than those of controls. Plasma total APN, HMW-APN, and HMWR values at discharge were 19.4 (7.2–27.3) μg/mL, 10.5 (3.2–12.8) μg/mL, and 0.52 (0.46–0.57), respectively, all of which were significantly decreased compared with the admission levels (Table 2 and Fig. 1). The plasma total APN value did not decrease to the degree of the controls.

Other variables also changed along with the treatment of HF (Tables 1 and 2). The body weight decreased by 6 kg. The BNP level was 677 (481–1206) pg/mL at admission and decreased to 212 (116–422) pg/mL at discharge. C-reactive protein levels, fasting glucose levels, and HOMA-R decreased. On the other hand, total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol levels increased.

Predictors of the total APN and HMW-APN

The plasma total APN levels at admission were positively related with age, but not with other factors known to relate with APN in previous reports, such as body weight, BNP, or HDL cholesterol (Table 3) [2,12,19–22]. On the other hand, the plasma total APN levels at discharge positively related with BNP and HDL cholesterol levels as well as age, and negatively related with mean blood pressure. Other factors known to relate with plasma APN in previous studies, such as alanine aminotransferase, triglyceride, creatinine, C-reactive protein, and hemoglobin levels did not relate with the plasma total APN levels throughout the study [20–24]. The echocardiographic variables did not relate with the plasma total APN levels.

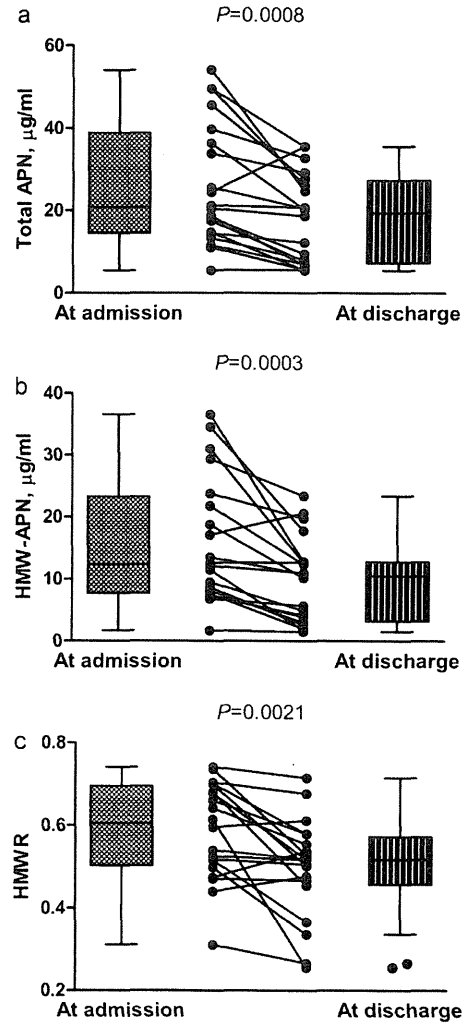

Figure 1 Changes of total adiponectin (APN) (a), high molecular weight APN (HMW-APN) (b), the ratio of HMW-APN to total APN (HMWR) (c) from admission to discharge.

The plasma HMW-APN levels positively related with age at admission and positively related with HDL and age at discharge (Table 4). We could not identify a significant predictor of HMWR at admission. The log-transformed plasma total APN value was the only predictor of the HMWR at the discharge ($\beta = 0.25$, $p = 0.003$). Women are known to have higher total APN, HMW-APN, and HMWR [3,19], however, we could not find sexual difference neither at admission nor at discharge (data not shown).

Prognosis

The mean follow-up period after the index admission was 302 days (range: 67–584 days). After the discharge, 3

Table 1 Patient characteristics.

	At admission	At discharge
Age, year	63 ± 11	
Male gender, *n* (%)	17 (85)	
Etiology, *n* (%)		
Dilated cardiomyopathy	10 (50)	
Dilated phase hypertrophic cardiomyopathy	4 (20)	
Hypertensive heart disease	3 (15)	
Others	3 (15)	
Physical examination		
Height, cm	163.8 ± 8.5	
Weight, kg	66.7 ± 11.9	60.2 ± 11.1*
Body mass index, kg/m^2	24.7 ± 3.3	22.3 ± 3.2*
Systolic blood pressure, mmHg	116 ± 17	110 ± 11
Diastolic blood pressure, mmHg	70 ± 14	64 ± 9
Mean blood pressure, mmHg	86 ± 13	79 ± 8
Heart rate, bpm	81 ± 22	67 ± 10*
NYHA functional class, *n* (%)		
II	6 (30)	
III	8 (40)	
IV	6 (30)	
Baseline medications, *n* (%)		
ACEI	7 (35)	10 (50)
ARB	9 (45)	8 (40)
Beta-blocker	13 (65)	19 (95)*
Diuretics	12 (60)	19 (95)*
Aldosterone blocker	5 (25)	13 (65)*
Digoxin	5 (25)	9 (45)
Nitrate	3 (15)	0 (0)
Oral inotropic agents	3 (15)	4 (20)
Acute phase treatment, *n* (%)		
Dopamine	1 (5)	
Dobutamine	5 (25)	
hANP	13 (65)	
Phosphodiesterase inhibitor	4 (20)	
Diuretics	13 (65)	
Laboratory examination		
AST, IU/L	31 (23–45)	26 (19–34)
ALT, IU/L	32 (19–51)	25 (15–45)
Total cholesterol, mg/dL	163 ± 36	194 ± 42*
Triglyceride, mg/dL	78 (66–115)	115 (96–148)*
HDL cholesterol, mg/dL	43 ± 10	50 ± 11*
Sodium, mEq/L	139 ± 3	138 ± 4
Blood urea nitrogen, mg/dL	21 ± 9	23 ± 12
Creatinine, mg/dL	1.0 ± 0.3	1.0 ± 0.3
Uric acid, mg/dL	7.5 ± 2.3	7.3 ± 2
C-reactive protein, mg/dL	0.45 (0.25–1.04)	0.12 (0.05–0.27)*
Fasting glucose, mg/dL	141 ± 48	104 ± 33*
Insulin, μU/mL	8.3 (5.9–15.0)	5.5 (3.8–13.6)
HOMA-R	2.5 (1.8–4.8)	1.3 (0.9–3.2)*
Hemoglobin, g/dL	13.0 ± 2.2	13.5 ± 2.3
Echocardiographic data		
Left ventricular end-diastolic diameter, mm	61 (53–69)	
Left ventricular end-systolic diameter, mm	52 (45–59)	
Fractional shortening, %	13 (12–19)	
Left atrial diameter, mm	53 (45–60)	
Interventricular septal wall thickness, mm	10 (7–11)	
Posterior wall thickness, mm	9 (8–11)	
Left ventricular mass, g	231 (204–281)	

Table 1 (*Continued*)

	At admission	At discharge
Left ventricular mass index, g/m^2	133 (117–176)	
E wave velocity, cm/s	86 (77–108)	
A wave velocity, cm/s	31 (22–36)	
E/A ratio	2.97 (2.19–3.93)	
Deceleration time, ms	130 (89–168)	
Mean Ea, cm/s	6 (5–7)	
Mean E/Ea ratio	16 (12–19)	

Continuous values are shown as median (interquartile range) or mean ± SD, as appropriate.
NYHA, New York Heart Association; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; hANP, human atrial natriuretic peptide; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment of insulin resistance; E, early diastolic transmitral flow; A, late diastolic transmitral flow; Ea, early diastolic mitral annular velocity.
* Significantly changed from admission to discharge ($p < 0.05$ with paired t-test, Wilcoxon's matched pairs signed rank test, or McNemar test as appropriate).

Table 2 Total APN, HMW-APN, and HMWR.

	Control ($n = 10$)	Heart failure ($n = 20$)		p^*
		Admission	Discharge	
Total APN, μg/mL	7.1 (5.3–10.0)	20.8 (14.5–38.9)†	19.4 (7.2–27.3)†	0.0008
HMW-APN, μg/mL	3.8 (2.7–5.5)	12.4 (7.7–23.3)†	10.5 (3.2–12.8)	0.0003
HMWR	0.52 (0.39–0.68)	0.61 (0.50–0.69)	0.52 (0.46–0.57)	0.0021
BNP, pg/mL	17 (6–27)	677 (481–1206)†	212 (116–422)†	0.0001

APN, adiponectin; HMW, high molecular weight; HMWR, HMW-APN ratio to total APN; BNP, B-type natriuretic peptide.
* Wilcoxon signed rank test comparing the values at admission and discharge.
† $p < 0.025$ vs. control compared with Mann–Whitney test.

Table 3 Predictors of total APN.

	At admission		At discharge	
	β	p	β	p
Age	0.01	0.023	0.02	0.002
Weight	−0.01	0.090	−0.01	0.157
Mean blood pressure	0.00	0.539	−0.02	0.022
Log BNP	0.43	0.076	0.33	0.037
Log ALT	0.00	0.576	0.13	0.577
Log triglyceride	0.00	0.985	−0.59	0.132
HDL-cholesterol	0.01	0.068	0.02	0.003
Creatinine	0.12	0.566	0.19	0.467
HOMA-R	−0.36	0.134	0.16	0.520
C-reactive protein	0.02	0.798	0.02	0.636
Hemoglobin	0.00	0.885	−0.03	0.303
Echocardiographic variables[a]				
Left ventricular end-diastolic diameter	0.00	0.584	0.03	0.889
Fractional shortening	−0.01	0.268	0.04	0.884
Left ventricular mass index	0.00	0.244	0.39	0.092
Mean E/Ea	0.00	0.832	−0.32	0.341

Dependant variable is log-transformed total APN.
APN, adiponectin; β, standardized coefficient; BNP, B-type natriuretic peptide; ALT, alanine aminotransferase; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment of insulin resistance; E, early diastolic transmitral flow; Ea, early diastolic mitral annular velocity.
[a] Echocardiographic variables are the values at admission.

Table 4 Predictors of HMW-APN.

	At admission		At discharge	
	β	p	β	p
Age	0.01	0.047	0.02	0.005
Weight	−0.01	0.142	−0.01	0.181
Mean blood pressure	0.00	0.557	−0.02	0.061
Log BNP	0.55	0.059	0.37	0.075
Log ALT	−0.15	0.491	0.45	0.315
Log triglyceride	−0.04	0.911	−0.71	0.153
HDL-cholesterol	0.01	0.119	0.02	0.004
Creatinine	0.22	0.371	0.33	0.323
HOMA-R	−0.40	0.092	0.17	0.494
C-reactive protein	0.04	0.653	0.04	0.567
Hemoglobin	−0.01	0.665	−0.05	0.175
Echocardiographic variables[a]				
Left ventricular end-diastolic diameter	0.00	0.503	0.11	0.650
Fractional shortening	−0.01	0.217	0.07	0.782
Left ventricular mass index	0.00	0.371	0.42	0.068
Mean E/Ea	0.00	0.986	−0.50	0.120

Dependent variable is log-transformed HMW-APN.
HMW-APN, high molecular weight adiponectin; β, standardized coefficient; BNP, B-type natriuretic peptide; ALT, alanine aminotransferase; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment of insulin resistance; E, early diastolic transmitral flow; Ea, early diastolic mitral annular velocity.
[a] Echocardiographic variables are the values at admission.

patients died and 6 patients were readmitted for worsening of HF. Exploratory survival analysis was performed with the composite endpoint of cardiac death and readmission for worsening of HF. The total APN and HMW-APN levels at the time of admission or discharge had no significant impact on the prognosis (data not shown). A higher value of HMWR at admission but not at discharge was associated with a better prognosis (Fig. 2a). A larger decrease in HMWR from admission to discharge was associated with a better prognosis (Fig. 2b). The patients with a higher HMWR at admission had a larger decrease in HMWR in response to the treatment (Table 5). The patients with a larger decrease in HMWR tended to have a higher HMWR at admission and were more hypertrophic (Table 5).

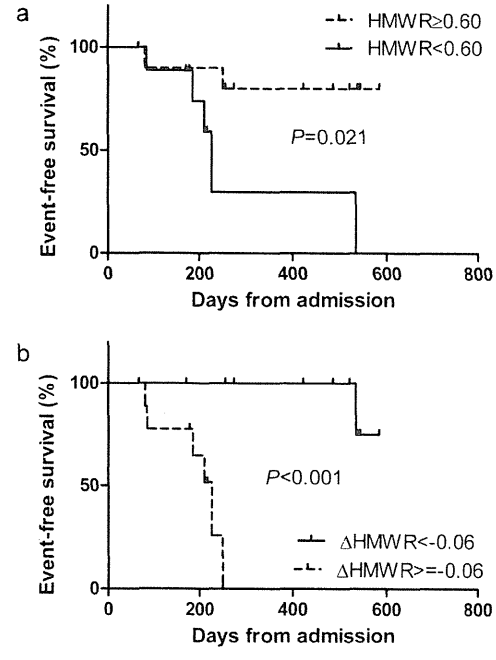

Figure 2 Kaplan–Meier event-free survival curves of groups divided by the ratio of high molecular weight adiponectin to total adiponectin (HMWR) at admission (a) and by the change of HMWR from admission to discharge (ΔHMWR) (b).

Discussion

This prospective observational study showed that the plasma total APN and HMW-APN were elevated at admission for exacerbation of acute HF. The plasma total APN, HMW-APN levels, and HMWR decreased in response to treatment. Furthermore, the patients with the higher HMWR at admission and those with the larger decrease in HMWR in response to the treatment showed a better prognosis.

Our study clarified the how plasma APN levels changed in the course of acute HF, which is consistent with a previous study showing that the total APN levels decreased during acute HF treatment [25]. Our study showed that the HMW-APN levels and HMWR as well as total APN decreased following acute HF treatment.

The plasma APN levels in acute HF may be modulated differently from in chronic HF. The plasma APN level is known to relate with gender, age, blood pressure, triglyceride, HDL cholesterol, renal function, liver function, hemoglobin, C-reactive protein, and BNP [2,12,19–24]. In this cohort, the plasma APN levels did not relate with these conventional predictors of APN other than age. The plasma total APN at

Table 5 Patient background by HMWR at admission and ΔHMWR.

	HMWR ≥ 0.60	HMWR < 0.60	*p*	ΔHMWR < −0.06	ΔHMWR ≥ −0.06	*p*
Age, year	63 ± 10	63 ± 12	0.984	60 ± 14	66 ± 6	0.231
Male	90%	80%	1.000	80%	90%	1.000
Body weight at admission, kg	68 ± 13	65 ± 11	0.594	69.8 ± 11.6	63.5 ± 11.9	0.244
Body weight at discharge, kg	62 ± 12	59 ± 10	0.598	63 ± 11.3	57.4 ± 10.7	0.271
Δbody weight, kg	−7 ± 3	−6 ± 3	0.874	−6.9 ± 3.1	−6.1 ± 3	0.598
Total APN at admission, mg/mL	29.1 (16.9 to 46.6)	17.8 (14.2 to 28.2)	0.226	19.6 (13.9 to 46.6)	23.4 (14.2 to 37.2)	0.940
Total APN at discharge, mg/mL	26.23 (6.9 to 33.5)	10.86 (7.2 to 20.55)	0.112	13.2 (6.4 to 29.7)	20.3 (9.1 to 26.9)	0.597
Δtotal APN, mg/mL	−6.1 (−14.9 to −3.8)	−6.9 (−11.3 to −1.8)	0.821	−9.5 (−14.9 to −3.8)	−5.1 (−10.5 to −1.8)	0.364
HMW-APN at admission, mg/mL	20.45 (10.65 to 31.9)	9.1 (7.1 to 14.15)	0.028	12.5 (8 to 31.9)	12.4 (7.1 to 22.3)	0.545
HMW-APN at discharge, mg/mL	12.7 (3.68 to 20)	5.3 (2.45 to 11.35)	0.082	7.4 (2.5 to 14.1)	10.6 (4.7 to 14.5)	0.545
ΔHMW-APN, mg/mL	−5.6 (−17.2 to −3.3)	−3.8 (−6.7 to −0.9)	0.199	−5.9 (−17.2 to −3.3)	−3.5 (−6.7 to −0.9)	0.112
HMWR at admission	0.69 (0.66 to 0.71)	0.51 (0.46 to 0.53)	<0.001	0.67 (0.59 to 0.7)	0.52 (0.46 to 0.62)	0.082
HMWR at discharge	0.54 (0.49 to 0.6)	0.49 (0.36 to 0.52)	0.131	0.51 (0.36 to 0.56)	0.52 (0.47 to 0.63)	0.364
ΔHMWR	−0.141 (−0.212 to −0.068)	−0.011 (−0.071 to 0.021)	0.005	−0.16 (−0.21 to −0.11)	−0.01 (−0.03 to 0.02)	<0.001
BNP at admission, pg/mL	853 (637 to 1320)	538 (396 to 1114)	0.226	707 (445 to 1083)	677 (502 to 1538)	0.650
BNP at discharge, pg/mL	160 (99 to 893)	278 (123 to 395)	0.705	160 (99 to 532)	270 (123 to 372)	0.650
ΔBNP, pg/mL	−516 (−679 to −190)	−445 (−902 to −67)	0.650	−516 (−679 to −139)	−445 (−902 to −172)	0.821
Fractional shortening, %	13 (12 to 17)	15 (11 to 21)	0.326	12 (10 to 17)	15 (13 to 21)	0.070
Left ventricular mass index, g/m^2	131 (109 to 152)	147 (117 to 189)	0.364	124 (97 to 137)	161 (125 to 189)	0.019

APN, adiponectin; HMW, high molecular weight; HMWR, HMW-APN ratio to total APN; BNP, B-type natriuretic peptide.