斎藤能彦	Asai O, Nakatani K, Tanaka T, Sakan H, Imura A, Yoshimoto S, Samejima KI, Yamaguchi Y, Matsui M, Akai Y, Konishi N, Iwano M, Nabeshima Y, Saito Y.	Decreased renal α-Klotho expression in early diabetic nephropathy in humans and mice and its possible role in urinary calcium excretion.	Kidney Int.	Epub ahead of print.	Jan 4.	2012
	Saito Y, Kishimoto I, Nakao K.	Roles of guanylyl cyclase-A signaling in the cardiovascular system.	Can J Physiol Pharmacol.	89	551-556	2011
	Saito Y.	Roles of atrial natriuretic peptide and its therapeutic use.	J Cardiol.	56	262-70	2010
矢野雅文	Suetomi T, Yano M, Uchinoumi H, Fukuda M, Hino A, Ono M, Xu X, Tateishi H, Okuda S, Doi M, Kobayashi S, Ikeda Y, Yamamoto T, Ikemoto N, Matsuzaki M.	Mutation-linked defective interdomain interactions within ryanodine receptor cause aberrant Ca^{2+}release leading to catecholaminergic polymorphic ventricular tachycardia.	Circulation	124(6)	682-694	2011
	Kobayashi S, Susa T, Tanaka T, Wada Y, Okuda S, Doi M, Nao T, Yoshiga Y, Yamada J, Okamura T, Ueyama T, Kawamura S, Yano M, Matsuzaki M.	Urinary 8-hydroxy-2'-deoxyguanosine reflects symptomatic status and severity of systolic dysfunction in patients with chronic heart failure.	Eur J Heart Fail. 2011	13(1)	29-36	2011
	Susa T, Kobayashi S, Tanaka T, Murakami W, Akashi S, Kunitsugu I, Okuda S, Doi M, Wada Y, Nao T, Yamada J, Ueyama T, Okamura T, Yano M, Matsuzaki M.	Urinary 8-hydroxy-2'-deoxyguanosine as a novel biomarker for predicting cardiac events and evaluating the effectiveness of carvedilol treatment in patients with chronic systolic heart failure.	Circ J	76(1)	117-126	2011
今泉勉	Oba T, Yasukawa H, Hoshijima M, Sasaki K-I, Futamata N, Fukui D, Mawatari K, Nagata T, Kyogoku S, Ohshima H, Minami T, Nakamura K, Kang D, Yajima T, Knowlton KU, Imaizumi T.	Cardiac-specific deletion of SOCS-3 prevents development of left ventricular remodeling after acute myocardial infarction.	J Am Coll Cardiol	59	838-852	2012
	Sugi Y, Yasukawa H, Kai H, Fukui D, Futamata N, Mawatari K, Oba T, Nagata N, Kyougoku S, Koga M, Imaizumi T.	Reduction and activation of circulating dendritic cells in patients with decompensated heart failure.	Int J Cardiol	147	258-264	2011
	Takayama N, Kai H, Kudo H, Yasuoka S, Mori T, Anegawa T, Koga M, Kajimoto H, Hirooka Y, Imaizumi T.	Simvastatin prevents large blood pressure variability induced aggravation of cardiac hypertrophy in hypertensive rats by inhibiting RhoA/Ras-ERK pathways.	Hypertens Res	34	341-347	2011

鄭忠和	Kusumoto A, Miyata M, Kubozono T, Ikeda Y, Shinsato T, Kuwahata S, Fujita S,Takasaki K, Yuasa T, Hamasaki S, Tei C.	Highly sensitive cardiac troponin T in heart failure: comparison with echocardiographic parameters and natriuretic peptides.	J Cardiol	59(2)	202-208	2012
	Fujita S, Ikeda Y, Miyata M, Shinsato T, Kubozono T, Kuwahata S, Hamada N, Miyauchi T, Yamaguchi T, Torii H, Hamasaki S, Tei C.	Effect of Waon therapy on oxidative stress in chronic heart failure.	Circ J	75(2)	348-356	2011
	Kuwahata S, Miyata M, Fujita S, Kubozono T, Shinsato T, Ikeda Y, Hamasaki S, Kuwaki T, Tei C.	Improvement of autonomic nervous activity by Waon therapy in patients with chronic heart failure.	J Cardiol	57(1)	100-106	2011
	Yoshimitsu M, Higuchi K, Miyata M, Devine S, Mattman A, Sirrs S, Medin JA, Tei C, Takenaka T.	Identification of novel mutations in the α-galactosidase A gene in patients with Fabry disease: pitfalls of mutation analyses in patients with low α-galactosidase A activity.	J Cardiol	57(3)	345-353	2011
	Yoshimitsu M, Higuchi K, Fan X, Takao S, Medin JA, Tei C, Takenaka T.	Sequencing and characterization of the porcine α-galactosidase A gene: towards the generation of a porcine model for Fabry disease.	Mol Biol Rep	38(5)	3145-3152	2011
	Niemann M, Breunig F, Beer M, Hu K, Liu D, Emmert A, Herrmann S, Ertl G, Wanner C, Takenaka T, Tei C, Weidemann F.	Tei index in fabry disease.	J Am Soc Echocardiogr	24(9)	1026-1032	2011
豊岡照彦	森田敏宏,豊岡照彦	高感度心筋トロポニンTによる心不全の予後予測	毎日メデイカルジャーナル	9	5-7	2011
竹石恭和	Yoshihisa A, Shimizu T, Owada T, Nakamura Y, Iwaya S, Yamauchi H, Miyata M, Hoshino Y, Sato T, Suzuki S, Sugimoto K, Yamaki T, Kunii H, Nakazato K, Suzuki H, Saitoh S, Takeishi Y	Adaptive servo ventilation improves cardiac dysfunction and prognosis in chronic heart failure patients with Cheyne-Stokes respiration.	Int Heart J	52 (4)	218-223	2011
	Ando K, Ishibashi T, Ohkawara H, Inoue N, Sugimoto K, Uekita H, Hu C, Okamoto Y, Takuwa Y, Takeishi Y	Crucial role of membrane type 1 matrix metalloproteinase (MT1-MMP) in RhoA/Rac1-dependent signaling pathways in thrombin-stimulated endothelial cells.	J Atheroscler Thromb	18 (9)	762-773	2011
	Kamioka M, Ishibashi T, Ohkawara H, Nagai R, Sugimoto K, Uekita H, Matsui T, Yamagishi S, Ando K, Sakamoto T, Takuwa Y, Wada I, Shiomi M, Maruyama Y, Takeishi Y	Involvement of membrane type 1-matrix metalloproteinase (MT1-MMP) in RAGE activation signaling pathways.	J Cell Physiol	226 (6)	1554-1563	2011

木村彰方	Arimura T, Ishikawa T, Nunoda S, Kawai S, Kimura A	Dilated cardiomyopathy-associated BAG3 mutations impair the Z-disc assembly and enhance the sensitivity to apoptosis in cardiomyocytes.	Hum Mutat	32(12)	1481-1491	2012
	Otsuka H, Arimura T, Abe T, Kawai H, Aizawa Y, Kubo T, Kitaoka H, Nakamura H, Nakamura K, Okamoto H, Ichida F, Ayusawa M, Nunoda S, Isobe M, Matsuzaki M, Doi YL, Fukuda K, Sasaoka T, Izumi T, Ashizawa N, Kimura A	Prevalence and distribution of sarcomeric gene mutations in Japanese patients with familial hypertrophic cardiomyopathy.	Circ J	76(2)	453-461	2012
	Purevjav E, Arimura T, Augustin S, Huby A-C, Takagi K, Nunoda S, Kearney DL, Taylor MD, Terasaki F, Bos JM, Ommen SR, Shibata H, Takahashi M, Itoh-Satoh M, McKenna W, Murphy RT, Labeit S, Yamanaka Y, Machida N, Park JE, Alexander PMA, Weintraub RG, Kitaura Y, Ackerman MJ, Kimura A, Towbin JA	Molecular basis for clinical heterogeneity in inherited cardiomyopathies due to myopalladin mutations.	Hum Mol Genet	21(9)	2039-2053	2012
今中恭子	Okamoto H, Imanaka-Yoshida K	Matricellular proteins: New molecular targets to prevent heart failure.	Cardiovasc Ther	Epub ahead of		2011
	Kobayashi N, Odaka K, Uehara T, Imanaka-Yoshida K, Kato Y, Oyama H, Tadokoro H, Akizawa H, Tanada S, Hiroe M, Fukumura T, Komuro I, Arano Y, Yoshida T, Irie T.	Toward in vivo imaging of heart disease using a radiolabeled single-chain fv fragment targeting tenascin-c.	Anal Chem	83	9123-9130	2011
	Ishigaki T, Imanaka-Yoshida K, Shimojo N, Matsushima S, Taki W, Yoshida T.	Tenascin-c enhances crosstalk signaling of integrin alphavbeta3/pdgfr-beta complex by src recruitment promoting pdgf-induced proliferation and migration in smooth muscle cells.	J Cell Physiol	226	2617-2624	2011
	Ando K, Takahashi M, Yamagishi T, Miyagawa-Tomita S, Imanaka-Yoshida K, Yoshida T, Nakajima Y.	Tenascin c may regulate the recruitment of smooth muscle cells during coronary artery development.	Differentiaion	81	299-306	2011
石坂信和	Ishizaka N, Sakamoto A, Imai Y, Terasaki F, Nagai R.	Multifocal fibrosclerosis and IgG4-related disease involving the cardiovascular system.	J Cardiol	59(2)	132-138	2012
	Kanzaki Y, Yamauchi Y, Okabe M, Terasaki F, Ishizaka N.	Three-dimensional architecture of cardiomyocytes and connective tissues in hypertrophic cardiomyopathy: a scanning electron microscopic observation.	Circulation	125(5)	738-739	2012
	Terasaki F, Tsuji M, Kizawa S, Fujita S, Kanzaki Y, Kitaura Y, Ishizaka N.	Sarcoidosis does not belong to or overlap with immunoglobulin G4-related diseases based on an assessment of serum immunoglobulin G4 levels in cardiac and noncardiac sarcoidosis.	Hum Pathol	Epub ahead of print		2012
植田初江	松山高明、池田善彦、植田初江	臓器移植の病理　心臓移植	病理と臨床	30(1)	15-22	2012

Ⅳ. 附録

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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

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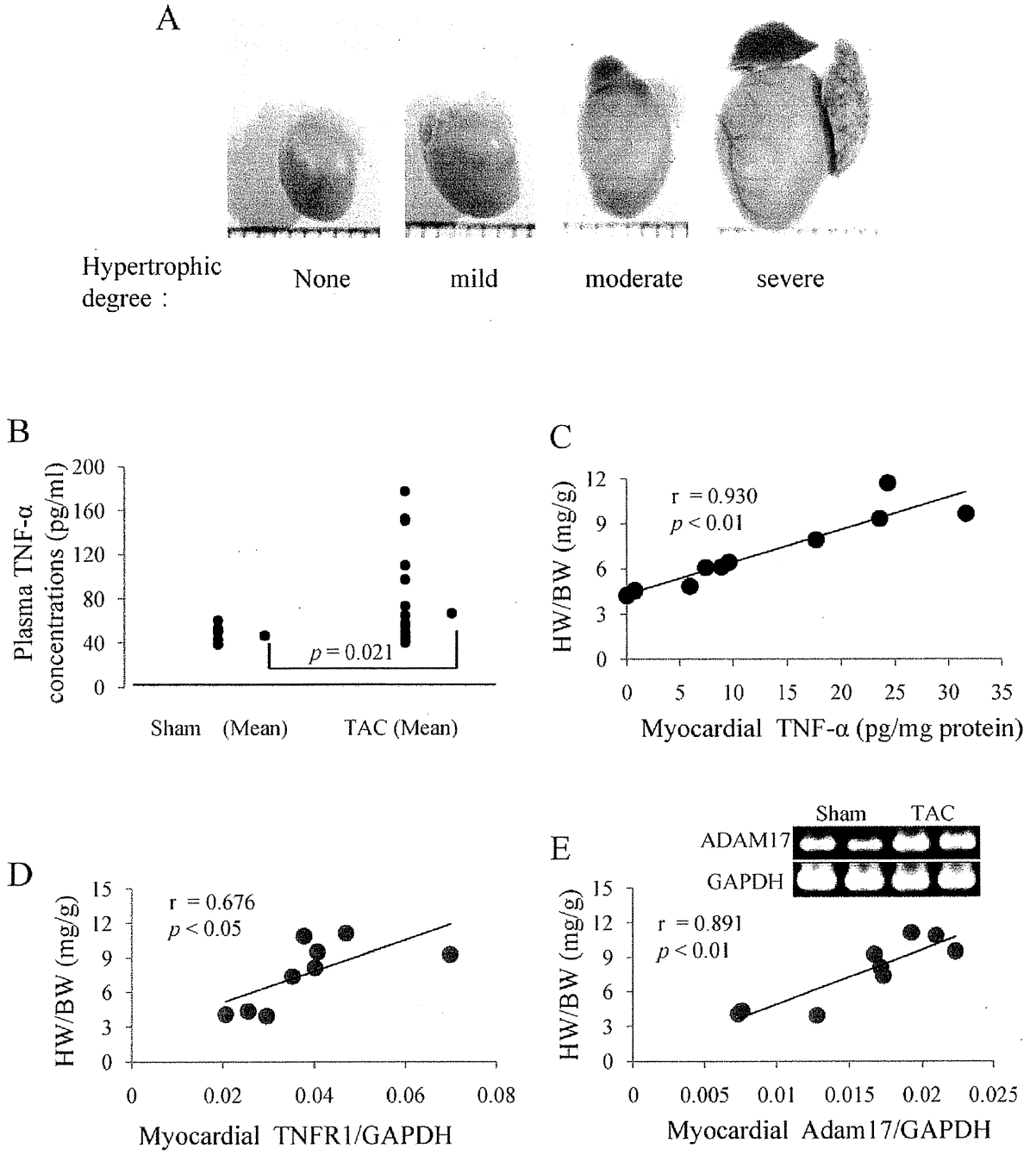

Fig. 1. Correlations between TNF-α signal molecules and cardiac hypertrophy. A. Representative pictures of whole hearts with various degree of hypertrophy. B. At 4 weeks after surgery, TNF-α concentrations were significantly higher in the TAC group ($n = 25$) than in sham group ($n = 9$). C. Correlation 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

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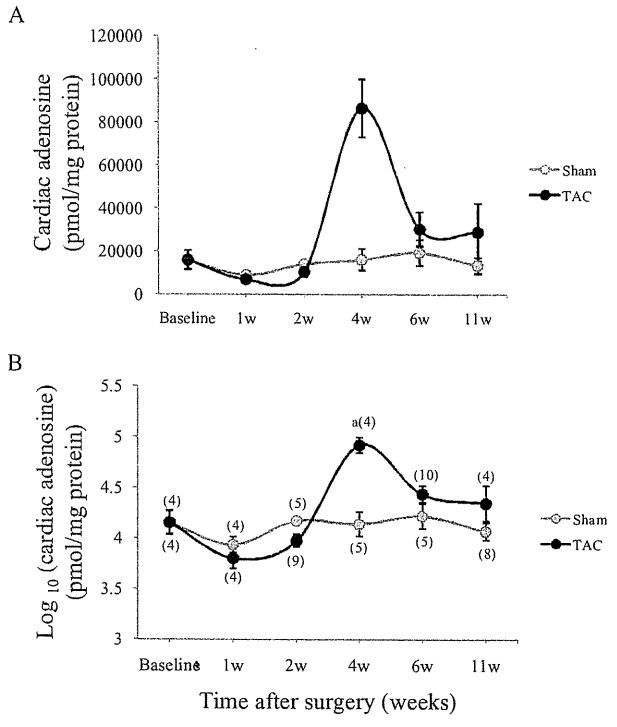

Fig. 2. Temporally changes of myocardial adenosine during cardiac hypertrophy development. Data in the panel A were transformed to logarithmic data as shown in the panel B. In TAC models, the cardiac adenosine level had a transient increase at 4 week after surgery ($^{a}P < 0.01$ vs the corresponding 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

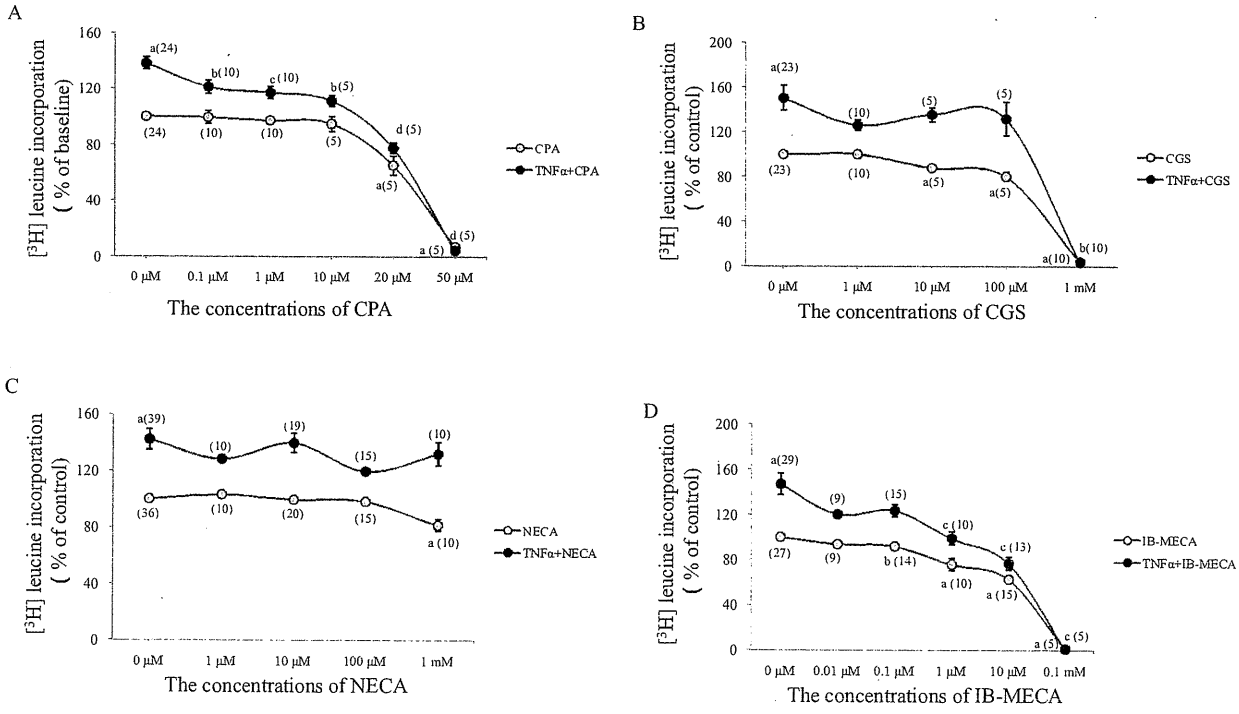

Fig. 3. Effect of adenosine agonists on TNF-α induced protein synthesis of cardiomyocytes. Incorporation of [^{3}H] leucine without addition of any agents (adenosine agonist 0 μM) was served as baseline. A. Safety range of CPA < 20 μM. $^{a}P < 0.001$ vs baseline; $^{b}P < 0.05$, $^{c}P < 0.01$ and $^{d}P < 0.010$ 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.

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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 down-stream 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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References

[1] Higuchi Y, Otsu K, Nishida K, et al. Involvement of reactive oxygen species-mediated NF-kappa B activation in TNF-alpha-induced cardiomyocyte hypertrophy. J Mol Cell Cardiol 2002;34:233–40.
[2] van Empel VP, De Windt LJ. Myocyte hypertrophy and apoptosis: a balancing act. Cardiovasc Res 2004;63:487–99.
[3] Stamm C, Friehs I, Cowan DB, et al. Inhibition of tumor necrosis factor-alpha improves postischemic recovery of hypertrophied hearts. Circulation 2001;104:I350–1355.
[4] Smith RM, McCarthy J, Sack MN. TNF alpha is required for hypoxia-mediated right ventricular hypertrophy. Mol Cell Biochem 2001;219:139–43.
[5] Stetson SJ, Perez-Verdia A, Mazur W, et al. Cardiac hypertrophy after transplantation is associated with persistent expression of tumor necrosis factor-alpha. Circulation 2001;104:676–81.
[6] Wang X, Oka T, Chow FL, et al. Tumor necrosis factor-alpha-converting enzyme is a key regulator of agonist-induced cardiac hypertrophy and fibrosis. Hypertension 2009;54:575–82.
[7] Takei Y, Di Tullio MR, Homma S, et al. Soluble tumor necrosis factor receptor 1 level is associated with left ventricular hypertrophy: the northern Manhattan study. Am J Hypertens 2009;22:763–9.
[8] Sriramula S, Haque M, Majid DS, Francis J. Involvement of tumor necrosis factor-alpha in angiotensin II-mediated effects on salt appetite, hypertension, and cardiac hypertrophy. Hypertension 2008;51:1345–51.
[9] Yokoyama T, Nakano M, Bednarczyk JL, McIntyre BW, Entman M, Mann DL. Tumor necrosis factor-alpha provokes a hypertrophic growth response in adult cardiac myocytes. Circulation 1997;95:1247–52.
[10] Meyer TE, Chung ES, Perlini S, et al. Antiadrenergic effects of adenosine in pressure overload hypertrophy. Hypertension 2001;37:862–8.
[11] Pang T, Gan XT, Freeman DJ, Cook MA, Karmazyn M. Compensatory upregulation of the adenosine system following phenylephrine-induced hypertrophy in cultured rat ventricular myocytes. Am J Physiol Heart Circ Physiol 2010;298:H545–53.
[12] Holycross BJ, Li P, Jackson EK. Adenosine-angiotensin II interactions, Part II. The role of adenosine in regulating angiotensin II-induced changes in heart rate and aldosterone release. J Pharmacol Exp Ther 1989;250:442–53.
[13] El-Ani D, Zimlichman R, Mashiach Y, Shainberg A. Adenosine and TNF-alpha exert similar inotropic effect on heart cultures, suggesting a cardioprotective mechanism against hypoxia. Life Sci 2007;81:803–13.
[14] Funakoshi H, Zacharia LC, Tang Z, et al. A1 adenosine receptor upregulation accompanies decreasing myocardial adenosine levels in mice with left ventricular dysfunction. Circulation 2007;115:2307–15.
[15] Villarreal F, Epperson SA, Ramirez-Sanchez I, Yamazaki KG, Brunton LL. Regulation of cardiac fibroblast collagen synthesis by adenosine: roles for Epac and PI3K. Am J Physiol Cell Physiol 2009;296:C1178–84.
[16] Dubey RK, Gillespie DG, Mi Z, Jackson EK. Endogenous cyclic AMP-adenosine pathway regulates cardiac fibroblast growth. Hypertension 2001;37:1095–100.
[17] Headrick JP, Peart JN, Reichelt ME, Haseler LJ. Adenosine and its receptors in the heart: regulation, retaliation and adaptation. Biochim Biophys Acta 2010;1808:1413–28.
[18] Funaya H, Kitakaze M, Node K, Minamino T, Komamura K, Hori M. Plasma adenosine levels increase in patients with chronic heart failure. Circulation 1997;95:1363–5.
[19] Kitakaze M, Minamino T, Node K, et al. Elevation of plasma adenosine levels may attenuate the severity of chronic heart failure. Cardiovasc Drugs Ther 1998;12:307–9.
[20] Wagner DR, Combes A, McTiernan C, Sanders VJ, Lemster B, Feldman AM. Adenosine inhibits lipopolysaccharide-induced cardiac expression of tumor necrosis factor-alpha. Circ Res 1998;82:47–56.
[21] Ke JJ, Yu FX, Rao Y, Wang YL. Adenosine postconditioning protects against myocardial ischemia-reperfusion injury though modulate production of TNF-alpha and prevents activation of transcription factor NF-kappaB. Mol Biol Rep 2011;38:531–8.
[22] Xu X, Fassett J, Hu X, et al. Ecto-5'-nucleotidase deficiency exacerbates pressure-overload-induced left ventricular hypertrophy and dysfunction. Hypertension 2008;51:1557–64.
[23] Liao Y, Takashima S, Asano Y, et al. Activation of adenosine A1 receptor attenuates cardiac hypertrophy and prevents heart failure in murine left ventricular pressure-overload model. Circ Res 2003;93:759–66.
[24] Zhao H, Liao Y, Minamino T, et al. Inhibition of cardiac remodeling by pravastatin is associated with amelioration of endoplasmic reticulum stress. Hypertens Res 2008;31:1977–87.
[25] Simpson P, McGrath A, Savion S. Myocyte hypertrophy in neonatal rat heart cultures and its regulation by serum and by catecholamines. Circ Res 1982;51:787–801.
[26] Asakura M, Kitakaze M, Takashima S, et al. Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. Nat Med 2002;8:35–40.
[27] Yamane R, Nakamura T, Matsuura E, Ishige H, Fujimoto M. A simple and sensitive radioimmunoassay for adenosine. J Immunoassay 1991;12:501–19.
[28] Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation 2003;107:3133–40.
[29] Monden Y, Kubota T, Inoue T, et al. Tumor necrosis factor-alpha is toxic via receptor 1 and protective via receptor 2 in a murine model of myocardial infarction. Am J Physiol Heart Circ Physiol 2007;293:H743–53.
[30] Fassett JT, Xu X, Hu X, et al. Adenosine regulation of microtubule dynamics in cardiac hypertrophy. Am J Physiol Heart Circ Physiol 2009;297:H523–32.
[31] Hill JA, Karimi M, Kutschke W, et al. Cardiac hypertrophy is not a required compensatory response to short-term pressure overload. Circulation 2000;101:2863–9.
[32] Esposito G, Rapacciuolo A, Naga Prasad SV, et al. Genetic alterations that inhibit in vivo pressure-overload hypertrophy prevent cardiac dysfunction despite increased wall stress. Circulation 2002;105:85–92.

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Original article

The utility of echocardiographic evaluation of donor hearts upon the organ procurement for heart transplantation

Shuji Hashimoto (MT)[a], Tomoko S. Kato (MD, PhD)[b,c,*], Kazuo Komamura (MD, PhD, FJCC)[b,c], Akihisa Hanatani (MD, PhD)[d], Kazuo Niwaya (MD, PhD)[e], Toshihiro Funatsu (MD, PhD)[f], Junjiro Kobayashi (MD, PhD)[g], Yoshiyuki Sumita (MT)[a], Norio Tanaka (MT)[a], Kazuhiko Hashimura (MD)[b,h], Masanori Asakura (MD, PhD)[b], Hideaki Kanzaki (MD, FJCC)[b], Masafumi Kitakaze (MD, PhD, FJCC)[b]

[a] *Department of Clinical Physiology, National Cerebral and Cardiovascular Center, Osaka, Japan*
[b] *Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Osaka, Japan*
[c] *Department of Organ Transplantation, National Cerebral and Cardiovascular Center, Osaka, Japan*
[d] *Department of Cardiology, Osaka City University School of Medicine, Osaka, Japan*
[e] *Department of Surgery and Cardiology, Garatia Hospital, Osaka, Japan*
[f] *Department of Cardiovascular Surgery, Kansai Rosai Hospital, Osaka, Japan*
[g] *Department of Cardiovascular Surgery, National Cerebral and Cardiovascular Center, Osaka, Japan*
[h] *Department of Medicine, Yao Municipal Hospital, Osaka, Japan*

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KEYWORDS
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Outcome

Summary
Background: Evaluating donor heart as accurately as possible upon the organ procurement would help lead careful post-transplant heart management. Our institution (National Cerebral and Cardiovascular Center, Osaka, Japan) has sent a transplant cardiologist upon the organ procurement for evaluating a donor heart ever since our first case of heart transplantation.
Methods: Thirteen consecutive bedside echocardiograms obtained from donors upon the organ procurement and post-transplant echocardiograms obtained from their recipients were retrospectively reviewed. The impact of donor echocardiograms on their recipients' post-transplant time course was analyzed and both the donor echocardiographic parameters and their recipients' parameters within 1 week after the heart transplant were compared.

* Corresponding author at: Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan. Tel.: +81 6 6833 5012; fax: +81 6 6872 8160.
E-mail addresses: rinnko@sannet.ne.jp, rinnko@hsp.ncvc.go.jp (T.S. Kato).

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Results: Both the left ventricular end-diastolic diameter and the ventricular wall thickness of donors correlated well with those parameters of their corresponding recipients ($r^2 = 0.740$, $p < 0.0001$, $r^2 = 0.704$, $p < 0.0001$, respectively). The information on coronary flow of the donor heart with risk factors for ischemic heart disease was useful for judging the availability for heart transplantation. The information on the pre-existing localized wall motion abnormality of donor hearts was useful for ruling out a possibility of rejection and other causes of wall motion abnormality after transplantation. The mean time required for bedside echocardiography for the donor heart was only 3.7 min. None of the recipients either developed primary graft failure or required treatment for cellular rejection.

Conclusions: Detailed observation of donor hearts by bedside echocardiograms upon the organ procurement is of clinical benefit.

Introduction

Heart transplant (HTx) provides considerable survival benefits for patients with end-stage heart failure; however, HTx is available for only a small fraction of these patients due to donor shortages all over the world [1,2]. In particular, the number of HTx surgeries completed in Japan has been remarkably small compared to that in other countries [3], because the domestic legal issues in Japan severely limit the number of organ donations. The mean waiting period of Japanese HTx candidates was often more than 2 years and occasionally reached 4 years even after the introduction of ventricular assist device surgery [4]. Finally, the Organ Transplant Law in Japan was revised in July 2009. In light of the enforcement of the Revised Organ Transplant Law in Japan, the number of HTx surgeries in Japan is expected to greatly increase in the near future.

Up to the present, transplant physicians in Japan have made a considerable effort to increase the number of transplants in Japan under the circumstances of extremely severe donor shortage. It has been reported that adequate and optimal use of all possible donor organs would be mandatory to increase graft availability [5,6]. Fukushima et al. have previously described the distinctive strategies for the donor evaluation and the management system to maximize cardiac and lung donors in Japan [6]. Since November 2002, a transplantation medical consultant doctor, who is an expert transplant cardiothoracic surgeon, has been sent to the procurement hospital by the Japan Organ Transplant Network for the purpose of assessment of donor organ function, management of donor hemodynamics, and prevention of any possible infections before the arrival of the harvest teams [6]. Transplantation medical consultant doctors sent by the Japan Organ Transplant Network also perform echocardiography and abdominal ultrasound on potential donors in order to eliminate inadequate organs to be donated. Based on the information from the transplantation medical consultant doctors, each potential recipient center could decide whether or not its organ procurement team should be sent to the donor hospital. In addition, each transplant center in Japan has also made an effort to disseminate a concept of transplantation to the entire nation and energetically appeal to the community by accomplishing good outcomes of HTx [7]. Evaluating donor heart as accurately as possible upon the organ procurement would bring essential information to their recipient team, leading to delicate and careful post-transplant management of the heart. As of March 2010, 69 HTx surgeries have been performed in Japan, and among those 27 transplants (39.1% of all HTx cases performed in Japan) were performed at our institution, the National Cerebral and Cardiovascular Center (NCVC), Osaka, Japan. Our institution has sent both transplant cardiologist and echocardiographic specialist upon the organ procurement accompanied by a surgical team for evaluating a donor heart ever since our first case of HTx surgery [8]. In the present study, we reviewed the bedside echocardiograms at organ procurement obtained from 13 consecutive transplant recipients who were transplanted since 2005. This is the first detailed report describing the echocardiograms of donor hearts and their impact on both transplant surgery and post-transplant management.

Methods

Patients and study design

The first HTx surgery at NCVC was performed in May 1999 [8]. Since then, 27 patients (39.1% of all HTx cases performed in Japan) have undergone HTx surgery as of March 2010 at NCVC [7]. We have sent both transplant cardiologist and echocardiographic specialist upon the organ procurement accompanied by a surgical team on all occasions of transplant surgery for graft evaluation and collecting information on the heart. Among those, we retrospectively reviewed 13 consecutive bedside echocardiograms on donor hearts at the time of organ procurement and the echocardiograms on their recipients recorded after HTx, whose HTx surgery has been performed since October 2005. We excluded the recipients who had HTx before October 2005 because we started to store the echocardiographic images of their donor hearts digitally for review and off-line analysis after HTx since October 2005.

Bedside echocardiography on potential donor hearts

Bedside echocardiography on a potential donor heart was performed using either the equipment at the donor hospital or our own Vivid I system (GE-Vingmed Ultrasound AS, Horten, Norway). We brought our portable echocardiographic machine (Vivid I system) to the donor hospital

for evaluating donor heart upon the organ procurement. Standard echocardiographic parameters, the area and distribution of ventricular wall motion abnormality, the presence or absence of atrial/ventricular septal defect, the presence or absence of valvular disease including valvular calcification were evaluated. In addition, coronary artery flow velocity of the left anterior descending artery was measured if the donor had any risk factors for ischemic heart disease. Risk factors for ischemic heart disease were defined as a current existence or past history of hyperlipidemia, hypertension, diabetes, or smoking, and older than 50 years of age. The standard echocardiographic examination included measurements of thickness of the ventricular septum and left ventricular (LV) posterior wall, and end-systolic and end-diastolic LV diameters from M-mode or 2-D imaging. LV ejection fraction was calculated by biplane Simpson's method from apical 4- and 2-chamber views [9]. Mitral inflow was obtained by pulsed-wave Doppler echocardiography with the sample volume between mitral leaflet tips during diastole, and peak early (E) and late (A) transmitral filling velocities, their ratio (E/A) and deceleration time of E were measured. Early diastolic annular velocity (E') was obtained by placing a tissue Doppler sampling volume at the septal and lateral mitral annulus in the apical 4 chamber view, and the E/E' ratio was calculated in a subset of 7 donors, whose bedside echocardiograms at the time of procurement were recorded using a machine equipped for tissue Doppler imaging. All donors were brain dead and on mechanical ventilation and with minimum dose of catecholamine and vasopressin for stabilization of hemodynamics.

Echocardiography on recipients' heart after transplant surgery

We performed scheduled echocardiography on HTx recipients at least more than once a week until 1 month of the heart transplantation, or when rejection/any hemodymamic changes were suspected. Post-HTx echocardiographic evaluation was performed using Vivid 7 (GE-Vingmed Ultrasound AS) with a M3S transducer. We used post-transplant echocardiographic measurements recorded within a week from their transplant surgery for comparison with the measurements obtained from their donors at the time of procurements. At the time of image acquisition, all recipients were in a stable hemodynamic condition with minimum dose or none of inotropic support.

Assessment of the impact of donor's bedside echocardiography on transplantation

The clinical time course of each patient during early postoperative period regarding development of rejection or graft dysfunction was retrospectively reviewed. The diagnosis of cellular acute rejection was based on the conventional International Society for Heart and Lung Transplantation (ISHLT) criteria [10]. Antibody-mediated rejection was defined in this study as positive staining for C4d at the capillary in the biopsy specimen, with or without hemodynamic deterioration [11,12]. The correlation between echocardiographic measurements obtained from the donor hearts and those from their recipients was evaluated.

Statistical analysis

Data are presented as means ± SD. The correlation between echocardiographic measurements obtained from bedside echocardiography at the time of organ procurement and measurements obtained from their recipients measured after HTx surgery was determined with the use of Pearson's correlation coefficient and the interclass correlation coefficient (ICC). The correlation between degree of valvular disease or wall motion abnormality obtained from donor and that of recipient was analyzed by Spearman's rank correlation coefficient. All statistical analyses were performed using JMP6.0 software (SAS Institute, Cary, NC, USA).

Results

Table 1 summarizes the demographic and clinical data from the studied patients. The primary disease that needs HTx was non-ischemic cardiomyopathy for all the patients. The recipients' age ranged from 19.5 to 51.0 years, whereas the donors' age ranged from 21.3 to 59.2 years. The ischemic time of donor hearts ranged from 166 to 235 min. Seven donors (53.8%) had risk factors for ischemic heart disease including 4 smokers.

None of the recipients developed primary graft dysfunction or hemodynamic compromise in the early postoperative period. None of the patients developed cellular rejection of ISHLT grade 3a or severer rejections (revised grading of Grade 2R [13]), although two patients developed antibody-mediated rejection requiring either steroid pulse therapy, intravenous immunoglobulin, or plasmapheresis. Two donor hearts were found to have arterial septal defect at the time of donor heart procurement, which was fixed during transplant surgery.

Table 2 summarizes the comparison of echocardiographic parameters between the results obtained from donors and their recipients. The LV ejection fraction was excellently preserved in all recipients (range 60.7–79.6%, mean 70.2 ± 6.0%). The LV diameters obtained from the donors were well correlated with the LV diameters obtained from their recipients (Fig. 1a). The LV mass index of the donor was also well correlated with the LV diameters obtained from their recipients. Based on the information on the donor heart size expressed as LV diameter, the transplant team made a medical decision as to whether the heart was of proper size for the candidate recipient who would be receiving the heart. Especially when the mismatch of donor–recipients body mass index was greater than 20% [14], we focused attention on the echocardiographic results of donor heart size for considering the appropriateness of the recipient selection. Indeed, we have performed the transplant surgery in 2 male recipients although their donors were female with the height of more than 20 cm shorter and body mass index of about 25% smaller than the recipients, according to the donor echocardiographic evaluation indicating sufficient heart size. For recipients who received a small heart based on the bedside donor echocardiographic evaluation at the time of

Table 1 Patients' characteristics and outcome.

Clinical characteristics of patients before heart transplantation	
Age at HTx (years)	40.4±9.1
Male (%)	8 (61.5%)
Mean BMI (kg/m^2)	19.8±2.0
Mean BSA (m^2)	1.54±0.1
LVAD implantation (%)	12 (92.3%)
Reason for HTx	
Idiopathic DCM	10 (78.2%)
Cardiac sarcoidosis	2 (13.0%)
ARVC	1(0%)
Donor information	
Donor age (years)	40.4±10.8
Mean BSA (m^2)	1.58±0.2
Donor heart ischemic time (min)	210.8±19.4
Risk factor for ischemic heart disease (%)	7 (53.8%)
Donor and recipient sex (donor → recipient)	
Male → male	6 (46.1%)
Male → female	2 (15.3%)
Female → male	2 (15.3%)
Female → female	3 (23.1%)
Rejection within 2 weeks and coronary artery disease at the first angiogram	
Number of patients with rejection of ≥ ISHLT grade 2	0(0%)
Number of patients with antibody mediated rejection	2 (15.3%)
Angiographically apparent coronary stenosis	3 (23.1%)
MIT of 0.5 mm on IVUS at the first study	4 (30.7%)

HTx, heart transplant; BMI, body mass index; BSA, body surface area; LVAD, left ventricular assist device; DCM, dilated cardiomyopathy; ARVC, arrhythmogenic right ventricular cardiomyopathy; ISHLT, International Society for Heart and Lung Transplantation; MIT, maximum intimal thickness; IVUS, intravascular ultrasound.

organ procurement including the above-described 2 male recipients, we had paid considerable attention to hemodynamics after HTx surgery such as gradual diminution of inotropic support and/or relatively longer duration of pressure monitoring with Swan-Ganz catheter. The information about ventricular wall thickness of the donor hearts correlated well with the ventricular thickness of their recipients measured after transplant as well (Table 2, Fig. 1b). According to the donor heart information about ventricular hypertrophy, which could be safely used in the heart transplantation to expand the donor pool [15], we administered cardioprotective agents, such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and calcium antagonists as soon as possible after HTx surgery for recipients who were preliminarily known to have received hypertrophic hearts. We experienced 2 patients with antibody mediated rejection in the early postoperative period, who showed a relatively increased LV wall thickness and LV mass at the time of rejection. We could suspect a possibility of antibody mediated rejection even without evidence of cellular rejection according to the information on their donor LV wall thickness. In these cases, donor bedside echocardiography was very useful to consider the timing of rejection treatment and/or further rejection surveillance.

The degree of valvular regurgitation of the donor heart did not always correspond with the degree of that seen in their recipients. In addition, peak early (E) and late (A) transmitral filling velocities and their ratio (E/A) (Fig. 1c), and deceleration time of E obtained from donors and their recipients were not corresponding with each other, either. Neither the E' nor the E/E' of the donor hearts were in agreement with the parameters measured in their recipients after HTx surgery.

The left anterior descending coronary artery flow was recorded in 7 donors with risk factors for ischemic heart disease, but they did not show a sign of significant organic coronary obstruction [16,17]. The mean diastolic-to-systolic coronary flow velocity ratio was greater than 1.4 for all 7 donor hearts with the risk factors [16], thus we decided to perform proceeding HTx surgery with the use of the donor hearts based on this information. For the patients with risk factors for ischemic heart disease, the first angiogram to evaluate coronary artery disease of the donor was performed 6 weeks from the HTx surgery, ahead of our routine schedule of 3 months after the transplants. None of the patients who received a donor heart with ischemic risk factors showed significant coronary artery stenosis greater than 75% at the time of first angiography. However, 2 patients whose donor hearts showed mean diastolic-to-systolic coronary flow velocity ratio of 1.4 were revealed to have about 50% stenosis on left anterior descending coronary artery at their first angiogram. They were then treated with intensive medication to prevent progression of transplant coronary artery disease including administration of a maximum dose of statin or/and early conversion to everolimus therapy [18–20]. Representative recording of the coronary flow obtained from a donor heart, whose recipient showed coronary irregularity at the first angiogram after the HTx surgery is shown in Fig. 2.

Table 2 Echocardiographic parameters obtained from the donors and their recipients.

Parameter	Donor echocardiogram ($n=13$)	Recipient echocardiogram ($n=13$)	ICC
Vital signs at the time of echocardiography			
Day of echocardiography performed	3.4±1.2 h before harvest surgery	3.7±1.6 days after HTx surgery	–
Systolic blood pressure (mm Hg)	117.5±13.5	109.6±7.8	–
Diastolic blood pressure (mm Hg)	78.8±11.9	72.6±10.4	–
Heart rate (bpm)	92.0±2.5	91.0±5.6	–
Conventional echocardiographic parameters			
LVDd (mm)*	39.1±3.5	40.3±3.8	*0.86*
LVDs (mm)	24.8±2.9	22.6±3.4	*0.79*
IVST (mm)*	10.0±1.5	12.0±1.6	*0.94*
PWT (mm)*	11.1±1.9	11.1±1.8	*0.95*
LVMI (g/m^2)*	113.0±21.5	110.6±29.7	*0.87*
LVEF (%)	62.3±6.4	70.2±6.0	*0.25*
TMF *E* (cm/s)	66.5±15.4	78.8±16.5	*0.53*
TMF *E/A*	1.7±0.6	2.1±0.6	*0.42*
TMF DcT (ms)	199.4±30.3	161.4±35.0	*0.32*
Tissue Doppler echocardiographic parameters (subgroup of 7 patients studied)			
E/E′ (septal) (cm/s)	7.6±2.7	8.1±2.4	*0.57*
E/E′ (lateral) (cm/s)	12.1±2.2	11.3±3.1	*0.20*
Left anterior descending coronary artery flow (subgroup of 7 patients studied)			
Diastolic-to-systolic flow velocity ratio*	1.6±0.1	1.7±0.2	*0.79*
Other findings (number of patients, %)			
Septal defect	2 (15.3%)	– (fixed)	–
Mitral valve calcification	0 (0%)	0 (0%)	–
Aortic valve calcification	0 (0%)	0 (0%)	–
Mild to moderate mitral regurgitation	2 (15.3%)	2 (15.3%)	*0.46*
Mild to moderate tricuspid regurgitation	2 (15.3%)	3 (23.1%)	*0.49*
Localized wall motion abnormality*	3 (23.1%)	4 (30.7%)	*0.89*

ICC, interclass correlation coefficient; HTx, heart transplant; LV, left ventricular; LVDd, LV end-diastolic internal dimension; LVDs, LV end-systolic internal dimension; IVST, thickness of the interventricular septum; PWT, thickness of the LV posterior wall; LVMI, LV mass index; LVEF, LV ejection fraction; TMF, transmitral flow; DcT, deceleration time of *E*.

* $p<0.001$ by correlation analysis between the values obtained from donors and their recipients.

Three donor hearts showed a localized wall motion abnormality at the apex. Both area and distribution of the wall motion abnormality found in each donor heart were also seen at the identical area and distribution in each recipient heart. This concordance of localized wall motion abnormality between donor and recipients meant that the wall motion abnormality of the recipient in the early postoperative period was not a newly developed abnormality which was unlikely caused by rejection or primary graft dysfunction. Accordingly, transplant physicians could just observe the changes in serial echocardiographic findings without doing any emergent tests to rule out the cause of the wall motion abnormality.

The time required for acquisition of images of bedside echocardiogram for donor heart evaluation was 3.7±1.3 min.

Discussion

In the present study, we have demonstrated the utility of bedside echocardiographic evaluation of donor hearts upon organ procurement. These are (a) the size of donor hearts measured by bedside echocardiography was in good agreement with the heart size measured after HTx surgery obtained from their recipients, which seems to be useful information for the judgment of proceeding with HTx especially in case of donor–recipient size mismatch; (b) the wall thickness of donor hearts derived from bedside echocardiography was also in good agreement with the wall thickness of their recipients measured after HTx surgery, and this information helped transplant physicians to optimize post-transplant medical therapy for the recipients who received hypertrophic hearts; (c) the information concerning septal defect would be of help for surgeons to prospectively plan the additional procedure of septal closure at the time of HTx surgery; (d) the information on coronary flow in left anterior descending artery of the donor heart with some risk factors for ischemic heart disease was useful for judging the availability of the heart, as well as for considering post-HTx medical management; (e) the information on pre-existing localized wall motion abnormality of donor hearts detected by bedside echocardiography was useful to consider a possibility of rejection and other causes of wall motion abnormality; (f) the mean time required for bedside echocardiography on donor heart was only 3.7 min, and this duration was reasonable for donor hospital and other organ team to accept our noninvasive evaluation of the graft.

We speculate that the discordances between the parameters of *E*, *E/A*, *E′* and *E/E′*, and the degree of valvular

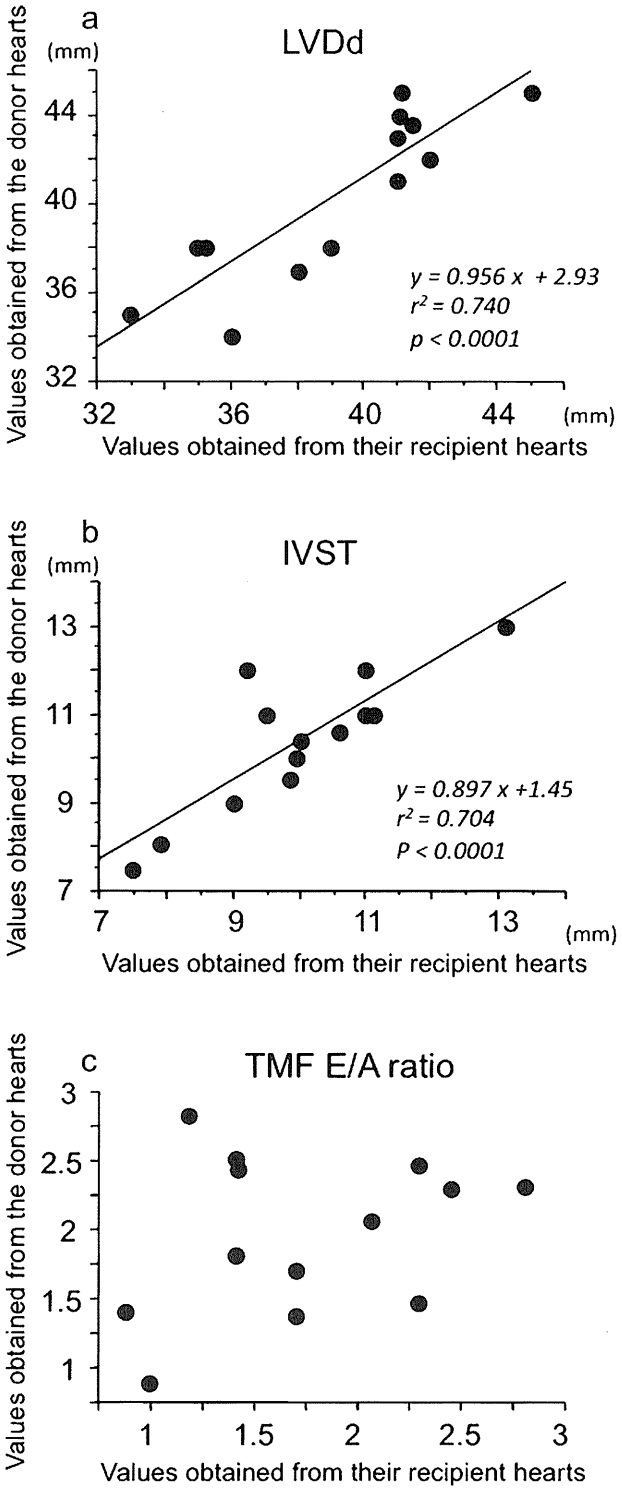

Figure 1 Correlations of the echocardiographic parameters between the value obtained from the donor at the time of organ procurement and the value obtained from their recipient within a week from transplant surgery regarding left ventricular end-diastolic diameter (LVDd) (a), ventricular septal wall thickness (IVST) (b), and the ratio of peak early and late transmitral filling velocities (TMF *E/A* ratio) (c). Closed circles represent the variables obtained from the same hearts before and after heart transplant surgery. Solid line indicates the correlation of variables obtained from donors and their recipients.

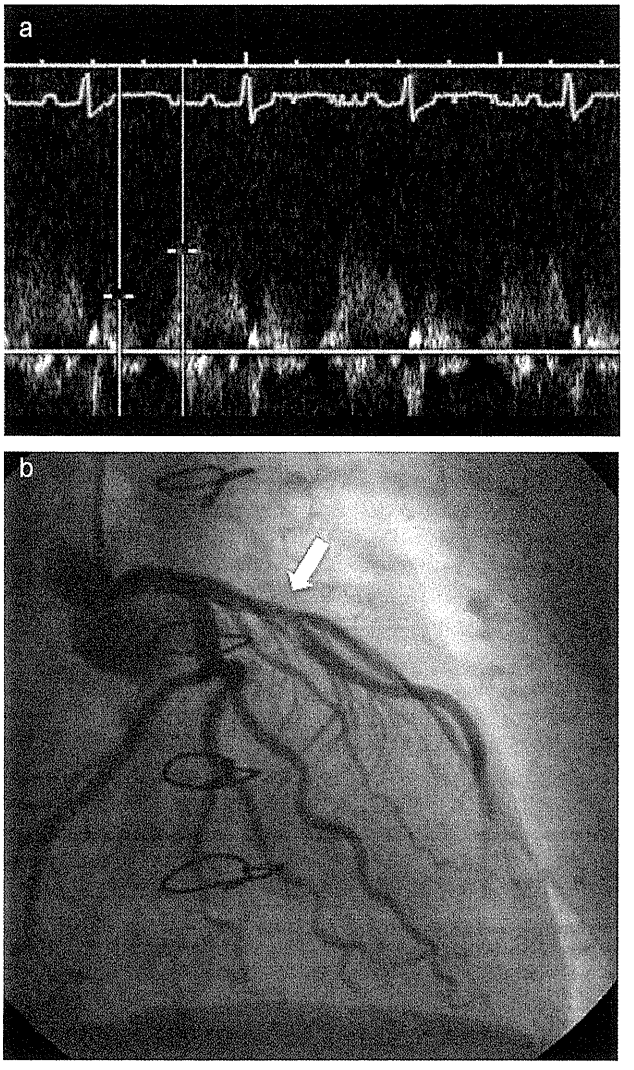

Figure 2 Representative recording of Doppler flow imaging of distal left anterior descending coronary artery (a) obtained from a donor heart with risk factors for ischemic heart disease, and the first angiogram of the recipient who received the heart which was performed in the month after transplant surgery (b). The arrow indicates about 50% narrowing of left anterior descending coronary artery.

regurgitation between the donors and their recipients were attributable to the difference of volume status between pre- and post-transplant. These Doppler-derived parameters were subjected to the preload status [21]; therefore, abnormality of these parameters in donor hearts would not be a factor influencing the decision of the heart availability.

One of the main focuses of bedside echocardiography on potential donor hearts is to determine the appropriateness of the use of the hearts for donation. In Japan, a transplantation medical consultant doctor who is sent by the Japan Organ Transplant Network preliminarily performs echocardiography on a potential donor to eliminate inadequate hearts to be donated, before the organ procurement team is sent to the donor hospital. Thus, we, as an organ procurement team, who have known which patient is going to receive the heart, perform more detailed

bedside echocardiography on the donor in the light of our potential recipient's detailed information upon the organ procurement. We actually decided not to harvest the donor heart only once during this study period according to our own bedside echocardiography, although the information from a transplantation medical consultant doctor about the heart was adequately enough to use the organ. The donor heart was relatively small for our potential recipient and showed decreased coronary flow and localized right heart wall motion abnormality upon the organ procurement; on the other hand our potential recipient had moderately high pulmonary vascular resistance and needed to receive a donor heart with good right ventricular function. That was why we decided not to harvest the donor heart. We immediately reported our decision and the results of our bedside echocardiography to the Organ Transplant Network.

Although this is a single-center retrospective analysis of small numbers of HTx donors and their recipients' echocardiograms, our institution is the biggest HTx center in Japan where so far 40% cases of HTx surgeries were performed. Thus, our endeavor to bring sufficient information on the donor hearts upon organ procurement to the transplant surgeons and physicians who were in charge of the recipients' post-transplant care for the purpose of successful outcome of HTx would bear mentioning. There had been a considerable effort by Fukusima et al. for maximizing the heart and lung transplant opportunities in Japan, such as the system of a special transplantation consultant doctor being sent to the donor hospital for evaluating the donor organs and stabilizing donor hemodynamics before the arrival of the harvest teams [6]. In addition to such efforts of maximizing the possible donor pool, each transplant center in Japan also has a responsibility of evaluating the donor heart. A good outcome after HTx surgery would be a key factor for undertaking transplantation in Japan. The survival rate of Japanese HTx recipients after HTx surgery has been excellent even compared to that in the International Heart Transplant Registry [7]. The excellent survival of Japanese recipients seems to be based on their excellent compliance with drugs and routine clinical visits due to full coverage of healthcare services provided by the National Health Insurance System; additionally, we believe that it was also due to the outstanding Japanese transplant physicians' endeavor. In order to gather as much information as possible, also in order to limit the evaluation time to be as short as possible, our institution sent our cardiac transplant cardiologist and echocardiographer to the donor hospital, who were experienced in HTx.

The limitation of this study was that the echocardiographic parameters obtained from the same hearts before and after HTx were recorded by different echocardiographic machines. Another limitation of this study was that we did not consider the dose of inotropic support before and after HTx when we compared the echocardiographic parameters. In addition, we could not report the detailed information on donors' demographics because of privacy protection in relation to personal data and because of the extremely small number of transplants in Japan. We did not have any pediatric cases in this observation, because the Previous Organ Transplant Law in Japan prohibited brain-death tests under 15 years old. That may be a reason we could show statistical correlations between donors and their recipients in the value of LV size and LV mass. In a decade of pediatric transplants that will be performed under the Revised Organ Transplant Law, then body-size mismatch would be a more important determinant in organ procurement. Thus, evaluation of LV size and LV mass by echocardiograms would be more necessary.

Despite several limitations, we still believe that we have achieved an excellent result of HTx recipients to be proud of [7] due to our significant effort of evaluating donor heart as carefully as possible and our strategy is worthwhile reporting. The number of HTx surgeries is expected to increase in the near future in Japan under the Revised Organ Transplant Law. Therefore, we hope that all transplant physicians in Japan find our detailed observation of donor hearts by echocardiograms reported here is of clinical benefit.

In conclusion, bedside echocardiogram upon organ procurement by transplant center's own staff is a unique system around the world, but we believe that it played a great role in improving the outcome after HTx.

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References

[1] Taylor DO, Stehlik J, Edwards LB, Aurora P, Christie JD, Dobbels F, Kirk R, Kucheryavaya AY, Rahmel AO, Hertz MI. Registry of the International Society for Heart and Lung Transplantation: Twenty-sixth Official Adult Heart Transplant Report-2009. J Heart Lung Transplant 2009;28:1007–22.

[2] Jaski BE, Kim JC, Naftel DC, Jarcho J, Costanzo MR, Eisen HJ, Kirklin JK, Bourge RC, Cardiac Transplant Research Database Research Group. Cardiac transplant outcome of patients supported on left ventricular assist device vs. intravenous inotropic therapy. J Heart Lung Transplant 2001;20:449–56.

[3] Oda N, Kato TS, Komamura K, Patel J, Kobashigawa JA. Retrospective review of Japanese patients undergoing heart transplantation in Japan compared with those undergoing transplantation in the United States. J Heart Lung Transplant 2010;29:1076–8.

[4] Osada K, Imaizumi T, Heart Transplant Candidate Registry Committee of the Japanese Circulation Society. Special report from the heart transplant candidate registry committee in Japan. J Heart Lung Transplant 2005;24:810–4.

[5] Wittwer T, Wahlers T. Marginal donor grafts in heart transplantation: lessons learned from 25 years of experience. Transpl Int 2008;21:113–25.

[6] Fukushima N, Ono M, Nakatani T, Minami M, Konaka S, Ashikari J. Strategies for maximizing heart and lung transplantation opportunities in Japan. Transplant Proc 2009;41:273–6.

[7] Oda N, Kato TS, Komamura K, Hanatani A, Mano A, Hashimura K, Asakura M, Niwaya K, Funatsu T, Kobayashi J, Wada K, Hashimoto S, Ishibashi-Ueda H, Nakano Y, Kihara Y, et al. Clinical course and outcome of heart transplant recipients: single center experience at National Cardiovascular Center in Japan. Int Heart J 2010;51:264–71.

[8] Kitamura S, Nakatani T, Yagihara T, Sasako Y, Kobayashi J, Bando K, Minatoya K, Hanatani A, Komamura K, Imakita M, Yutani C, Kuro M, Kimura K, Nonogi H, Miyatake K. Cardiac transplantation under new legislation for organ transplantation in Japan: report of two cases. Jpn Circ J 2000;64:333–9.

[9] Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. J Am Soc Echocardiogr 1989;2:358–67.

[10] Billingham ME, Cary NR, Hammond ME, Kemnitz J, Marboe C, McCallister HA, Snovar DC, Winters GL, Zerbe A. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart Rejection Study Group. The International Society for Heart Transplantation. J Heart Transplant 1990;9:587–9.

[11] Wu GW, Kobashigawa JA, Fishbein MC, Patel JK, Kittleson MM, Reed EF, Kiyosaki KK, Ardehali A. Asymptomatic antibody-mediated rejection after heart transplantation predicts poor outcomes. J Heart Lung Transplant 2009;28:417–22.

[12] Gupta S, Mitchell JD, Lavingia B, Ewing GE, Feliciano MN, Kaiser PA, Ring WS, Stastny P, Patel PC, Markham DW, Mammen PP, Dimaio JM, Drazner MH. Utility of routine immunofluorescence staining for C4d in cardiac transplant recipients. J Heart Lung Transplant 2009;28:776–80.

[13] Stewart S, Winters GL, Fishbein MC, Tazelaar HD, Kobashigawa J, Abrams J, Andersen CB, Angelini A, Berry GJ, Burke MM, Demetris AJ, Hammond E, Itescu S, Marboe CC, McManus B, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. J Heart Lung Transplant 2005;24:1710–20.

[14] Chen JM, Sinha P, Rajasinghe HA, Suratwala SJ, McCue JD, McCarty MJ, Caliste X, Hauff HM, John R, Edwards NM. Do donor characteristics really matter? Short- and long-term impact of donor characteristics on recipient survival, 1995–1999. J Heart Lung Transplant 2002;21:608–10.

[15] Goland S, Czer LS, Kass RM, Siegel RJ, Mirocha J, De Robertis MA, Lee J, Raissi S, Cheng W, Fontana G, Trento A. Use of cardiac allografts with mild and moderate left ventricular hypertrophy can be safely used in heart transplantation to expand the donor pool. J Am Coll Cardiol 2008;51: 1214–20.

[16] Higashiue S, Watanabe H, Yokoi Y, Takeuchi K, Yoshikawa J. Simple detection of severe coronary stenosis using transthoracic Doppler echocardiography at rest. Am J Cardiol 2001;87:1064–8.

[17] Tani T, Tanabe K, Kitai T, Yamane T, Kureha F, Katayama M, Tamita K, Kaji S, Oda T, Ehara N, Kinoshita M, Yamamuro A, Morioka S, Kihara Y. Detection of severe stenosis and total occlusion in the left anterior descending coronary artery with transthoracic Doppler echocardiography in the emergency room. Echocardiography 2009;26:15–20.

[18] Kobashigawa JA. Statins and cardiac allograft vasculopathy after heart transplantation. Semin Vasc Med 2004;4:401–6.

[19] Kato T, Tokoro T, Namii Y, Kobayashi T, Hayashi S, Yokoyama I, Morimoto S, Chan M, Giannetti N, Hunt SA. Early introduction of HMG-CoA reductase inhibitors could prevent the incidence of transplant coronary artery disease. Transplant Proc 2000;32:331–3.

[20] Eisen HJ, Tuzcu EM, Dorent R, Kobashigawa J, Mancini D, Valantine-von Kaeppler HA, Starling RC, Sørensen K, Hummel M, Lind JM, Abeywickrama KH, Bernhardt P, RAD B253 Study Group. Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. N Engl J Med 2003;349:847–58.

[21] Kato TS, Noda A, Izawa H, Yamada A, Obata K, Nagata K, Iwase M, Murohara T, Yokota M. Discrimination of nonobstructive hypertrophic cardiomyopathy from hypertensive left ventricular hypertrophy on the basis of strain rate imaging by tissue Doppler ultrasonography. Circulation 2004;110: 3808–14.

Clinical Investigations

Increased Left Atrial Volume Index Predicts a Poor Prognosis in Patients With Heart Failure

HARUTOSHI TAMURA, MD,[1] TETSU WATANABE, MD,[1] SATOSHI NISHIYAMA, MD,[1] SHINTARO SASAKI, MD,[1] TAKANORI ARIMOTO, MD,[1] HIROKI TAKAHASHI, MD,[1] TETSURO SHISHIDO, MD,[1] TAKEHIKO MIYASHITA, MD,[1] TAKUYA MIYAMOTO, MD,[1] JOJI NITOBE, MD,[1] OSAMU HIRONO, MD,[2] AND ISAO KUBOTA, MD[1]

Yamagata, Japan

ABSTRACT

Background: Left atrial volume index (LAVI) is known to reflect the duration and severity of increased left atrial pressure caused by left ventricular (LV) diastolic dysfunction. However, the prognostic value of LAVI in patients with heart failure (HF) has not been fully investigated.

Methods and Results: Transthoracic echocardiography was performed in 146 consecutive patients (78 men, 68 women; mean age 72 ± 12 y) who were hospitalized for HF. There were 45 cardiac events (32%) during a median follow-up period of 448 days. There were no significant differences in LV end-diastolic dimensions or ejection fraction between patients who did or did not have cardiac events. However, LAVI was markedly higher in patients with, than those without, cardiac events (56 ± 26 vs 44 ± 22 mL/m^2; $P < .01$). Kaplan-Meier analysis showed that there was a stepwise increase in risk of cardiac events with each increment of LAVI category, and LAVI >53.3 mL/m^2 correlated with the highest risk of cardiac events (log-rank test; $P < .01$). Multivariate Cox proportional hazard analysis showed that high LAVI was an independent predictor for cardiac events (hazard ratio 1.427; 95% confidence interval 1.024–1.934; $P < .05$).

Conclusion: LAVI may be useful for stratification of risk in patients with HF. (*J Cardiac Fail 2011;17:210–216*)

Key Words: Diastolic dysfunction, left atrial volume, risk stratification, prognostic factor.

Heart failure (HF) is a major cause of death, and it has a poor prognosis despite the significant reduction in mortality achieved in clinical trials.[1–3] Therefore, the prognostic evaluation and stratification of risk in patients with HF continue to be important, involving complex assessments of multiple interacting variables. Numerous studies have shown that left ventricular (LV) systolic dysfunction and diastolic dysfunction are prognostic factors for HF.[4,5]

Because the left atrium (LA) is directly exposed to LV diastolic pressure through the mitral valve, the size of the LA reflects the duration and severity of increased LA pressure following increased LV diastolic pressure. Therefore, LA volume is reported to be a sensitive marker of LV diastolic dysfunction.[6–8] Recently, the LA volume index (LAVI) was suggested as a new marker for cardiac function. It was also reported that a high LAVI was a powerful predictor of poor prognosis after acute myocardial infarction.[9] However, the prognostic value of LAVI in patients with HF has not been fully investigated.

The aim of the present study was to examine the clinical significance of LAVI in patients with HF. We hypothesized that LAVI increases with increasing severity of HF, and that LAVI provides important prognostic information.

From the [1]Department of Cardiology, Pulmonology, and Nephrology, Yamagata University School of Medicine, Yamagata, Japan and [2]Yamagata Prefectural Shinjo Hospital, Yamagata, Japan.

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Reprint requests: Tetsu Watanabe, MD, PhD, Department of Cardiology, Pulmonology, and Nephrology, Yamagata University School of Medicine, 2-2-2 Iida-Nishi, Yamagata 990-9585, Japan. Tel: +81-23-628-5302; Fax: +81-23-628-5305. E-mail: tewatana@med.id.yamagata-u.ac.jp

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Methods

Study Design

Out of 166 consecutive patients with HF, we prospectively studied 146 patients (78 men, 68 women; mean age 72 ± 12 y) who were admitted to Yamagata University Hospital, Yamagata, Japan, for treatment of worsening HF or therapeutic evaluation of HF. The diagnosis of HF was made by 2 senior cardiologists using the generally accepted Framingham criteria[10] and other relevant information, including a history of dyspnea and symptomatic exercise intolerance with signs of pulmonary congestion or peripheral edema, the presence of moist rales on auscultation, or documentation of left ventricular enlargement or dysfunction by chest X-ray or echocardiography.[11]

The functional severity of HF at admission was assessed as New York Heart Association (NYHA) functional class II in 34 patients, class III in 73 patients, and class IV in 39 patients. The etiology of HF was dilated cardiomyopathy in 40 patients (27%), ischemic heart disease in 37 (25%), valvular heart disease in 19 (13%), hypertensive heart disease in 17 (12%), and other causes in 33 (23%). Diagnoses of hypertension, diabetes, and hyperlipidemia were obtained from medical records or patient histories of current or previous medical therapy. Patients with no history of atrial fibrillation (AF), and who did not show AF on continuous electrocardiographic monitoring during hospitalization, were defined as patients with sinus rhythm, and patients with transient and chronic AF were defined as AF patients. The exclusion criteria were renal insufficiency characterized by a serum creatinine concentration >2.0 mg/dL (n = 10), severe mitral regurgitation (MR; n = 4) or previous mitral valve surgery (n = 4), mitral stenosis (n = 1), and atrioventricular block (n = 1). Informed consent was given by each of the patients before participation in the study, and the protocol was approved by the institution's Human Investigations Committee.

Blood samples were obtained at admission and discharge for measurement of plasma B-type natriuretic peptide (BNP), creatinine, uric acid, and sodium. Plasma BNP levels were measured by using a commercially available specific radioimmunoassay (Shiono RIA BNP assay kit; Shionogi Co, Tokyo, Japan).[11] Clinical data, including age, gender, and NYHA functional class at admission, were obtained from hospital medical records and patient interviews. Diuretics were administered in flexible doses on the basis of body weight and daily diuresis. The time of discharge was decided by 2 senior cardiologists.

Echocardiography

Transthoracic echocardiography was performed 3–7 days before discharge, on a Hewlett-Packard Sonos 7500 ultrasound instrument, equipped with a sector transducer (carrier frequency of 2.5 or 3.75 MHz). Therefore, all echocardiographic data were measured at the chronic compensation phase of HF.

LA volume was assessed at LV end-systole by using the biplanar area-length method from 4- and 2-chamber views.[7] Measurements of LA volume were indexed by body surface area (LA volume index; LAVI). The normal range for LAVI has been reported to be 14–26 mL/m^2.[12,13] An LAVI value ≥32 mL/m^2 is considered to indicate significant enlargement,[7] and an LAVI value ≥40 mL/m^2 is considered to indicate severe enlargement.[14]

Left atrial dimension (LAD) was measured at end-systole in the 2-dimensional parasternal long-axis view. LV internal diameter and wall thickness were measured at end-diastole and end-systole in the 2-dimensional parasternal long-axis view.[14] LV end-diastolic dimension (LVDd) was used to calculate LV mass index (LVMI), using an anatomically validated formula.[15] LV ejection fraction (LVEF) was calculated using the biplanar method of disks (modified Simpson rule).[14] The Tei index was measured as previously described.[16] All patients underwent pulsed-wave Doppler examination of mitral inflow. Peak transmitral-flow E-wave and A-wave velocities, E-wave deceleration time (DCT), and the ratio of E-wave to A-wave were measured from the apical 4-chamber view. The apical 4-chamber view was used to obtain tissue Doppler imaging (TDI) of the mitral annulus. A sample volume of the pulsed-wave Doppler was positioned at the lateral side of the mitral annulus, and the spectral signal of the mitral annular velocity was recorded. The peak early (E′) mitral annular velocity was measured and the ratio of the E-wave to E′ (E/E′) calculated. All echocardiographic measurements were calculated as mean values from 5 consecutive cardiac cycles.

Endpoints and Follow-up of Patients

Patients were prospectively followed until the occurrence of cardiac events, and no patients were lost to follow-up after discharge (median follow-up period of 502 days). The endpoints were: 1) cardiac death, defined as death due to worsening HF or sudden cardiac death; and 2) worsening HF requiring readmission to hospital.[11,17] Sudden cardiac death was defined as death without definite preceding symptoms or signs and was confirmed by the attending physician.

Statistical Analysis

Results are expressed as mean ± SD for continuous variables and as percentages of the total number of patients for categoric variables. Skewed variables are presented as median and interquartile range. The *t* test and chi-square test were used for comparison of continuous and categoric variables, respectively. When the data was not normally distributed, the Mann-Whitney test was used. A Cox proportional hazard analysis was performed to determine independent predictors of cardiac events for the entire population. Variables that were significant in the univariate analysis were entered into the multivariate model which adjusted for age, LVDd, and AF. The cardiac event–free curve was analyzed by the Kaplan-Meier method and compared by the log-rank test. The optimum LAVI for predicting cardiac events was determined as that giving the largest sum of sensitivity plus specificity on the receiver operating characteristic (ROC) curve. ROC curves were constructed to evaluate the area under the curves (AUC). Statistical significance was defined as $P < .05$. Statistical analyses were performed using a standard statistics computer program. The intraobserver and interobserver reliability of LAVI measurements were assessed by 2 echocardiologists in 20 patients, each repeated once. Based on the intraclass correlation coefficient, the mean intraobserver reliability of LAVI measurements was 98.0% and the mean interobserver reliability was 95.6%.

Results

Clinical Characteristics of the Study Subjects

The mean age of the study subjects was 72 ± 12 years, 53% of the patients were men, 36% were classified as AF patients, and 77% were in NYHA functional classes III or

Table 1. Comparison of Clinical Characteristics Between Patients With and Without Cardiac Events

	Event Free (n = 101)	Cardiac Event (n = 45)	*P* Value
Age (y)	72 ± 12	73 ± 11	.4915
Gender (M/F)	49/52	29/16	.0748
NYHA functional class I-II/III-IV (at admission)	28/73	6/39	.0485
NYHA functional class I-II/III-IV (at discharge)	101/0	44/1	.1027
AF	35 (35%)	18 (40%)	.5350
Heart rate (beats/min)	66 ± 12	69 ± 13	.3177
Hypertension	63 (62%)	27 (60%)	.7851
Diabetes mellitus	36 (36%)	17 (38%)	.8044
Hyperlipidemia	36 (36%)	16 (36%)	.9918
Current smoking	47 (47%)	22 (49%)	.7925
Etiology of heart failure			.6859
DCM	31 (31%)	9 (20%)	
ICM	23 (23%)	14 (31%)	
VHD	13 (13%)	6 (13%)	
HHD	12 (12%)	5 (11%)	
Others	22 (22%)	11 (24%)	
Echocardiography			
LAD (mm)	42 ± 8	46 ± 8	.0106
LVDd (mm)	56 ± 21	53 ± 9	.4614
LVEF (%)	47 ± 15	43 ± 16	.1701
E/A (sinus rhythm)	0.85 ± 0.45	0.99 ± 0.73	.2231
Deceleration time (ms)	190 ± 57	182 ± 69	.4538
Tei index	0.56 ± 0.33	0.64 ± 0.37	.3884
MR moderate	13 (13%)	12 (27%)	.0361
LVMI (g/m^2)	201 ± 69	225 ± 81	.1098
E/E′	11.0 ± 6.2	13.8 ± 7.9	.0184
LAVI (mL/m^2)	44 ± 22	56 ± 26	.0037
Blood marker at admission			
BNP (pg/mL)	516 (266–1100)	765 (401–1375)	.0823
Log_{10} BNP	2.75 ± 0.44	2.86 ± 0.35	.1633
Serum creatinine (mg/dL)	0.92 ± 0.39	0.97 ± 0.38	.0244
Uric acid (mg/dL)	6.6 ± 1.9	6.8 ± 1.9	.0424
Sodium (mEq/L)	141 ± 4	140 ± 4	.3289
Blood marker at discharge			
BNP (pg/mL)	216 (88–399)	259 (129–447)	.0027
Log_{10} BNP	2.29 ± 0.47	2.56 ± 0.38	.0010
Medication			
ACE inhibitors or ARBs	88 (87%)	37 (82%)	.2871
β-blockers	67 (66%)	24 (53%)	.1039
Ca-channel blockers	26 (26%)	7 (16%)	.1401
Diuretics	90 (89%)	41 (91%)	.7129
Digitalis	20 (20%)	6 (13%)	.2182

AF, atrial fibrillation; DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; VHD, valvular heart disease; HHD, hypertensive heart disease; LAD, left atrial dimension; LVDd, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAVI, left atrial volume index; BNP, B-type natriuretic peptide; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker. Results are presented as mean ± SD, n (%), or median (interquartile range).

IV at admission. The etiology of HF was ischemic heart disease in 25% and nonischemic heart disease in 75%. The mean LVDd, LVEF, and LAVI were 56 mm, 46%, and 48 mL/m^2, respectively. The median plasma BNP level at admission was 583 pg/mL.

Follow-up was completed for all patients. There were 45 cardiac events (31%) during the follow-up period, and these comprised 7 cardiac deaths and 38 rehospitalizations for worsening HF.

The clinical characteristics of patients with or without cardiac events were compared (Table 1). NYHA functional class was worse in patients with cardiac events than in those without cardiac events. There were no significant differences in age, gender, prevalence of AF, hypertension, diabetes mellitus, hyperlipidemia, or etiology of HF between patients with or without cardiac events (Table 1).

Echocardiographic Parameters

LAD, E/E′, and LAVI were greater and MR more severe in patients with than in those without cardiac events (Table 1). There were no significant differences in other echocardiographic parameters between patients with and without cardiac events (Table 1).

Medication Use and Blood Markers at Admission

Serum creatinine and uric acid levels were significantly higher in patients with than without cardiac events (Table 1). Although there was no significant difference in plasma BNP levels at admission between patients with or without cardiac events, plasma BNP levels at discharge were significantly higher in patients with than without cardiac events. There were no significant differences in