Table 5 Subgroup analysis for all cause death according to eGFR levels

	Total patients, n	*All cause death*, n	*Odds ratio*	*95% CI*	*P-value*
Male	1632	39			
⩾60	997	6	1.00	Reference	
30–59	550	16	1.55	0.83–2.92	0.17
<30	85	17	4.60	1.81–11.70	<0.01
Female	634	71			
⩾60	307	24	1.00	Reference	
30–59	260	28	1.76	0.61–5.08	0.30
<30	67	19	10.23	3.43–34.52	<0.001
Age ⩾65 years	1327	91			
⩾60	597	23	1.00	Reference	
30–59	610	39	1.45	0.81–2.60	0.21
<30	120	29	4.13	1.68–10.14	<0.01
Age <65 years	939	19			
⩾60	707	7	1.00	Reference	
30–59	200	5	2.40	0.60–9.68	0.22
<30	32	7	27.12	6.70–78.28	<0.001
Hypertension	1010	70			
⩾60	471	14	1.00	Reference	
30–59	429	30	2.02	0.98–4.19	0.06
<30	110	26	7.33	2.56–21.05	<0.001
No hypertension	1256	40			
⩾60	833	16	1.00	Reference	
30–59	381	14	1.13	0.50–2.56	0.76
<30	42	10	2.69	1.12–6.58	<0.05
Diabetes mellitus	513	44			
⩾60	254	8	1.00	Reference	
30–59	193	18	1.25	0.67–2.35	0.49
<30	66	18	10.66	2.66–42.73	<0.01
No diabetes mellitus	1753	66			
⩾60	1050	22	1.00	Reference	
30–59	617	26	2.68	0.99–5.66	0.06
<30	86	18	3.38	1.17–9.83	<0.05

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate.
Adjusted for demographic (age and sex), medical (body mass index, smoking, hypertension, diabetes mellitus, dyslipidemia, prior cardiovascular disease, Q wave myocardial infarction and peak creatine kinase), angiographic (number of diseased vessles and killip classification ⩾II) and procedural (mechanical support, TIMI (thrombolysis in myocardial infarction) flow grade 0 at admission and TIMI flow grade 3 after treatment) variables.

impact of eGFR in AMI and to determine whether it is independently associated with prognosis, we analyzed data from a prospective broad cohort of patients with AMI. The patients in the present study had baseline serum creatinine levels ranging from 0.3 to 12.6 mg dl^{-1}, and percutaneous coronary intervention was performed in 92.5% of the patients during the acute phase. We thus could extend the prognostic impact of CKD from selected patients in large-scale clinical trials to a diverse cohort of AMI patients in general. Kasai *et al.*[29] demonstrated that lower eGFR values were significant long-term predictors for all-cause and cardiac mortality in Japanese patients who underwent complete coronary revascularization using a 10-year cohort study. The present study also confirmed these findings. Although the present study included both patients that underwent complete and incomplete coronary revascularization, adjustments for this variable were included in the statistical analysis. As a result, the findings in this study might be more applicable to a general population.

The present study demonstrated that the significant risk factors differed depending on the outcomes such as all cause and cardiac death (Table 4). There are potential explanations for these findings. Cardiac death was defined as death due to heart failure, fatal arrhythmia, cardiac rupture and recurrent myocardial infarction. In contrast, all cause death included not only cardiac death but also other causes of death such as infection. Killip classification and prior cardiovascular disease were independent predictors for all cause death, suggesting that heart failure associated with AMI might induce systemic organ failure and other complications leading to death. Mechanical support was an independent predictor for cardiac death, indicating that severe circulatory shock associated with

AMI may result in cardiac death, including fatal arrhythmia and cardiac rupture.

There are several possible explanations by which CKD increases the in-hospital mortality in patients with AMI. CKD may be indicative of traditional risk factors such as older age, hypertension, dyslipidemia and diabetes mellitus, which have been established to be closely related to cardiovascular outcomes.[30] Therefore, CKD may reflect the presence of severe coronary artery disease. Even after adjustments for demographic, medical, angiographic and procedural variables, the eGFR remained a significant risk for in-hospital all-cause and cardiac mortality. The increase in mortality with a reduced eGFR can be partly explained by nontraditional factors associated with CKD, including increased inflammatory factor levels,[31] elevated homocysteine levels,[32] enhanced coagulability[31] and endothelial dysfunction,[33] which were not assessed in this study.

Study limitations

First, we only assessed the baseline eGFR and could not determine the effects of changes in eGFR on outcomes. Second, the present study did not collect data regarding the use of medication after hospitalization because it varies widely among patients according to their clinical status, especially during the acute phase of AMI. However, the use of medication may affect the outcomes of patients during the long-term follow-up. Third, information was not collected during the follow-up after discharge, and the impact of CKD on the long-term outcomes in AMI patients could not be assessed. Fourth, coronary catheterization was performed after the onset of AMI in all patients, but severe patients who could not undergo coronary catheterization were not included. Therefore, the present results cannot be applied to all patients with AMI in general.

CONCLUSION

The reduced eGFR was a significant and independent risk for in-hospital all-cause and cardiac mortality in a broad cohort of Japanese patients hospitalized with AMI. Evaluation of renal function and effective management of these high-risk patients with AMI is important.

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ORIGINAL ARTICLE

Plasma adiponectin levels predict cardiovascular events in the observational Arita Cohort Study in Japan: the importance of the plasma adiponectin levels

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As the plasma level of adiponectin is related to metabolic syndrome and cardiovascular events, a low plasma adiponectin level may either cause or trigger cardiovascular disorders. The purpose of this study was to determine whether a low adiponectin level contributes to cardiovascular events, and to investigate the factors influencing adiponectin in the Japanese Arita-cho cohort study.We followed about 2000 subjects in Arita-cho, Saga, Japan as a cohort study, and we enrolled 637 subjects (205 men; 65.1 ± 8.3 years old) who participated in annual health checks from 2005 to 2008 and underwent measurement of the plasma adiponectin level and an oral glucose tolerance test. We monitored the incidence of cardiovascular or cerebrovascular events in these subjects until the end of 2010, discontinuing follow-up at 3 years after the start of enrollment. Subjects with low plasma adiponectin levels ($<10.5\,ng\,ml^{-1}$) had a higher incidence of newly diagnosed cardiovascular diseases such as acute heart failure or acute myocardial infarction than those with high plasma adiponectin levels ($\geqslant 10.5\,ng\,ml^{-1}$) over an average of 2.95 years of follow-up. Multivariate analysis showed that the adiponectin level was predicted by the following parameters in all subjects: age ($\beta = 0.16$), male gender ($\beta = -0.267$), homeostasis model assessment of insulin resistance ($\beta = -0.140$) and the plasma levels of high-density lipoprotein cholesterol ($\beta = 0.104$), uric acid ($\beta = -0.13$), triglycerides ($\beta = -0.169$) and brain natriuretic peptide ($\beta = 0.151$). The difference in plasma glucose before and 120 min after the intake of a 75-g glucose load did not influence the plasma adiponectin level. The plasma adiponectin level is useful for predicting cardiovascular events, and is a measure of the risk of lifestyle-related diseases.
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Keywords: adiponectin; myocardial infarction; stroke

INTRODUCTION

Adiponectin is a protein that is secreted exclusively by adipose tissue and contributes to the regulation of lipid and glucose metabolism. Adiponectin is also reported to have anti-inflammatory and anti-atherosclerogenic effects[1,2] in addition to its influence on insulin sensitivity and hepatic gluconeogenesis.[3,4] Such reports have led to the concept that a low plasma adiponectin level could predict the incidence of vascular events. However, there have been few prospective studies of plasma adiponectin levels and coronary artery disease (CAD) in healthy populations, and the results obtained have been inconclusive. The British Women's Heart and Health Study found no association between the plasma adiponectin level and new-onset CAD during 4 years of follow-up.[5] In older Dutch subjects, a high baseline plasma adiponectin level was associated with increased 15-year mortality.[6] In young survivors (<60 years of age) following a first myocardial infarction, however, a low plasma adiponectin level was associated with recurrent infarction.[7] Indeed, hypoadiponectinemia has usually been recognized as a risk factor for CAD[8-10] and the adiponectin level was linked to the severity of heart failure in our previous study.[11] Taken together, it seems that the plasma adiponectin level may influence cardiovascular or cerebrovascular events but not decisively, because the adiponectin level varies in relation to gender, race and the presence or absence of lifestyle-related disease.

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Therefore, we investigated the predictive value of the plasma adiponectin level for cardiovascular or cerebrovascular disease, and explored factors that influence adiponectin in a cohort study performed in Arita-cho (Saga, Japan). As adiponectin is closely related to the pathophysiology of diabetes mellitus, we particularly investigated the influence of insulin resistance, and that of the glucose and insulin levels after the oral glucose tolerance test (OGTT), on the plasma adiponectin level.

METHODS

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

Study population

The study population included participants in the health check program of Arita-cho (Saga, Japan) from 2005 to 2008 (the Arita-cho cohort Study). Participants who underwent an OGTT were included, but those who had cardiovascular and/or cerebrovascular disease at baseline were excluded from follow-up.

We enrolled 637 of the more than 2000 subjects who participated in the Arita-cho cohort study. The mean age, systolic/diastolic blood pressure (BP) and heart rate of the men ($n=205$) and women ($n=432$) was 66.4 ± 8.2 and 65.1 ± 8.4 years, 134.4 ± 18.0/82.6 ± 10.8 and 129.7 ± 20.0 mm Hg/78.8 ± 10.2 mm Hg and 63.3 ± 10.0 and 66.1 ± 10.1 per min, respectively.

Laboratory tests

Blood was collected from each participant at least 10 h after the last food intake to measure the plasma levels of adiponectin, fasting plasma glucose, total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and brain natriuretic peptide (BNP). The height, body weight and waist circumference of all participants were also measured. The OGTT was performed after a 10-h overnight fast. Each participant ingested a solution containing 75 g of dextrose, and venous blood samples were obtained at 0, 30, 60, 90 and 120 min for determination of the plasma glucose level, whereas plasma insulin levels were measured at 0 and 120 min. Then Δ insulin was calculated as the difference between the baseline and the 2-h insulin values, whereas ΔBG (Δ blood glucose) was calculated as the difference between the baseline and the 2-h plasma glucose values.

The plasma concentration of adiponectin was measured by an immunoradiometric assay and the plasma concentration of BNP was measured with a commercial kit immunoradiometric assay for human BNP (Shionoria; Shionogi, Osaka, Japan). The insulin resistance index was determined by the homeostasis model assessment of insulin resistance (HOMA-IR) method.

Cardiovascular and cerebrovascular events

Information on cardiovascular and cerebrovascular diseases was obtained from a standardized questionnaire at baseline and at the annual health checks. If a subject provided information that suggested the possibility of new-onset cardiovascular or cerebrovascular disease, we obtained confirmation from the clinic or hospital where the participant had consulted a cardiologist or neurologist. Diagnosis of new cardiovascular or cerebrovascular disease was defined as the study endpoint during follow-up. Cardiovascular events were defined as hospitalization or death due to heart failure or else the occurrence of acute myocardial infarction. Cerebrovascular events were defined as hospitalization or death due to stroke. For participants without cardiovascular or cerebrovascular events, the final date of follow-up was the date of last contact and follow-up was discontinued at 3 years after enrollment.

Statistical Analysis

Results are expressed as the mean ± s.d. or as percentages. Analysis of variance was employed for continuous variables and the χ^2-test was used for categorical data to compare differences between groups. Tukey's test was used for *post-hoc* analysis if analysis of variance revealed a statistically significant difference.

For variables that did not show a normal distribution, including TG, HOMA-IR, BNP and adiponectin, the data were transformed into natural logarithmic values before statistical analysis. Multivariate analysis was performed to identify independent predictors. Gender-specific multivariate linear regression models were used to identify the associations that remained significant after adjustment for other variables. Cardiovascular and cerebrovascular event-free curves were drawn by the Kaplan–Meier method and then were compared by the log-rank test. All analyses were performed with SPSS software (SPSS version 12.0, Chicago, IL, USA), and $P<0.05$ was considered statistically significant.

RESULTS

Among 637 subjects, 384 participants without history of either cardiovascular or cerebrovascular disease enrolled in Arita-cho from 2005 to 2007 completed follow-up. These participants were divided into two groups based on the 50th percentile of plasma adiponectin ($10.5\,ng\,ml^{-1}$). During the follow-up period, five participants from the low-adiponectin group ($<10.5\,ng\,ml^{-1}$) suffered from cardiovascular disease (acute decompensated heart failure due to hypertension in three and acute myocardial infarction in two) and four participants developed cerebrovascular disease (cerebral hemorrhage in two and cerebral infarction in two). In the high-adiponectin group ($\geqslant 10.5\,ng\,ml^{-1}$), two participants suffered from cerebrovascular disease (both had cerebral infarction) and none of the participants developed cardiovascular disease. The cumulative cardiovascular and cerebrovascular event-free rate was lower in the low-adiponectin group (Figure 1). The cardiovascular event-free rate was markedly decreased in the low-adiponectin group (Figure 2), whereas the cerebrovascular event-free rate was similar in both groups (Figure 3).

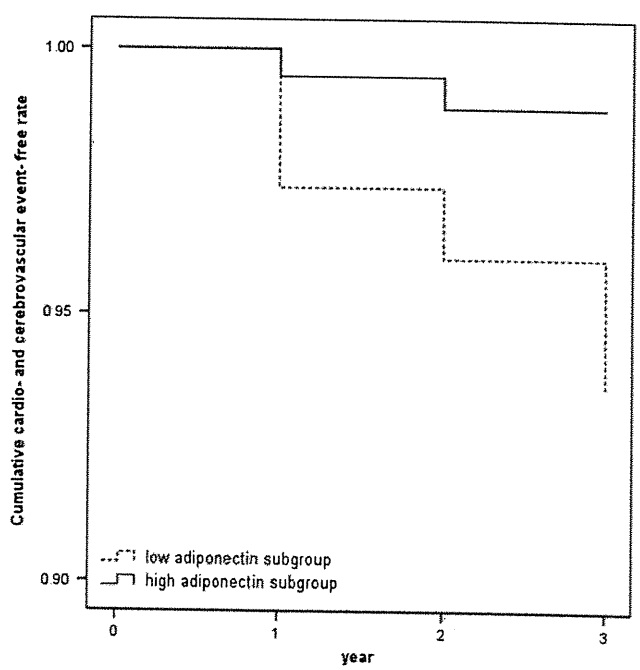


Figure 1 Cardiovascular and cerebrovascular event-free curves obtained with the Kaplan–Meier method in the respective groups divided by adiponectin levels. All participants were divided into two groups according to 50th percentile of adiponectin levels ($10.5\,ng\,ml^{-1}$). Cumulative cardiovascular and cerebrovascular event-free rates in the low-adiponectin subgroup ($n=191$) and high-adiponectin subgroup ($n=193$) were 93.6% and 98.9%, respectively (log-rank test, $P=0.02$).

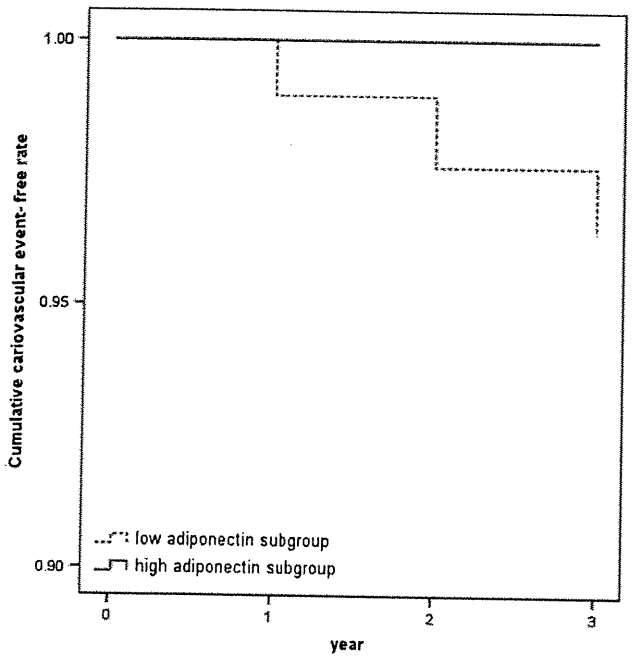


Figure 2 Cardiac event-free curves obtained with the Kaplan–Meier method in the respective groups divided by adiponectin levels. All participants were divided into two groups according to 50th percentile of adipoecntin levels (10.5 ng ml^{-1}). Cumulative cardiaovascular event-free rates in the low-adiponectin subgroup ($n=191$) and high-adiponectin subgroup ($n=193$) were 96.4% and 100%, respectively (log-rank test, $P=0.02$).

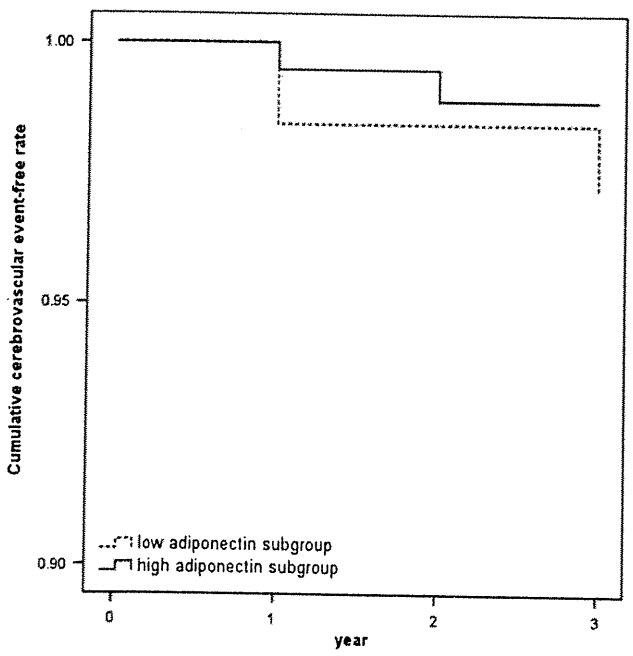


Figure 3 Cerebrovascular event-free curves obtained with the Kaplan–Meier method in the respective groups divided by adiponectin levels. All participants were divided into two groups according to 50th percentile of adipoecntin levels (10.5 ng ml^{-1}). Cumulative cerebrovascular event-free rates in the low-adiponectin subgroup ($n=191$) and high-adiponectin subgroup ($n=193$) were 97.2% and 98.9%, respectively (log-rank test, $P=0.37$).

Adiponectin level may be related to BP, and we found that four men in the low-adiponectin group developed hypertension, whereas none of the subjects in the high-adiponectin group developed hypertension, hinting that adiponectin is related to hypertension. However, systolic BP in the low- and high-adiponectin groups were 130.0 ± 18.6 and 131.0 ± 19.1 mm Hg, respectively ($P=0.40$); diastolic BPs in the low- and high-adiponectin group were 79.5 ± 9.5 and 78.2 ± 10.6 mm Hg, respectively ($P=0.26$). Furthermore, the systolic and diastolic BP during the annual check-up in three subjects who developed heart failure due to hypertension was 130.2 ± 20.0 and 81.2 ± 8.3 mm Hg, respectively, at examination. The two subjects with a low adiponectin level who had myocardial infarction had no experience of smoking, and did not have diabetes mellitus, but both had hypertension and one of them had dyslipidemia. Our data set did not include data for anticoagulants and anti-hypertensive therapy.

We then investigated factors that influenced the plasma adiponectin level. Table 1 shows the clinical characteristics of all 637 participants from 2005 to 2008 categorized by plasma adiponectin quartiles (first quartile, <6.66 ng ml^{-1}; second quartile, $\geqslant 6.66$ ng ml^{-1} and <9.80 ng ml^{-1}; third quartile, $\geqslant 9.80$ ng ml^{-1} and <14.28 ng ml^{-1}; fourth quartile: $\geqslant 14.28$ ng ml^{-1}). There were no significant differences of HbA_{1c} among the four quartiles. The fourth quartile was older than the other three quartiles, and plasma levels of HDL-C and BNP were significantly higher in the fourth quartile than in the other three. The fourth quartile had lower BMI, waist circumference, plasma uric acid, plasma TG, Δ insulin, Δ BG, HOMA-IR and percentage of men than the other three quartiles. The first quartile had higher fasting BG levels and a higher prevalence of diabetes mellitus than the other three quartiles. In addition, the mean BMI of the four quartiles was below 25. The plasma adiponectin level (Ln adiponectin) was higher in women than in men (2.42 ± 0.51 *vs.* 1.99 ± 0.99; $P<0.0001$). The plasma adiponectin level was negatively associated with HOMA-IR in women, men and all subjects (Figure 4).

To further clarify the factors that influenced the plasma adiponectin level, multivariate linear regression analysis was performed with plasma adiponectin as the dependent variable. As a result, the age ($\beta=0.16$), HOMA-IR ($\beta=-0.140$), male gender ($\beta=-0.267$) and plasma levels of HDL-C ($\beta=0.104$), uric acid ($\beta=-0.13$), TG ($\beta=-0.169$) and BNP ($\beta=0.151$) were significant predictors of the plasma adiponectin level in all participants (Table 2). Because women have higher plasma adiponectin levels than men, we separately investigated the determinants of adiponectin in men and women. We found that the age ($\beta=0.153$), HOMA-IR ($\beta=-0.208$) and plasma BNP($\beta=0.149$) were independently associated with the plasma adiponectin level in men, whereas the age ($\beta=0.194$), Δ insulin ($\beta=-0.152$), HOMA-IR ($\beta=-0.131$) and plasma levels of HDL-C ($\beta=0.129$), uric acid ($\beta=-0.119$), TG ($\beta=-0.179$) and BNP ($\beta=0.161$) were independently associated with adiponectin in women. Thus, the present study demonstrated that HOMA-IR, but not Δ BG, is associated with a decrease of the plasma adiponectin level.

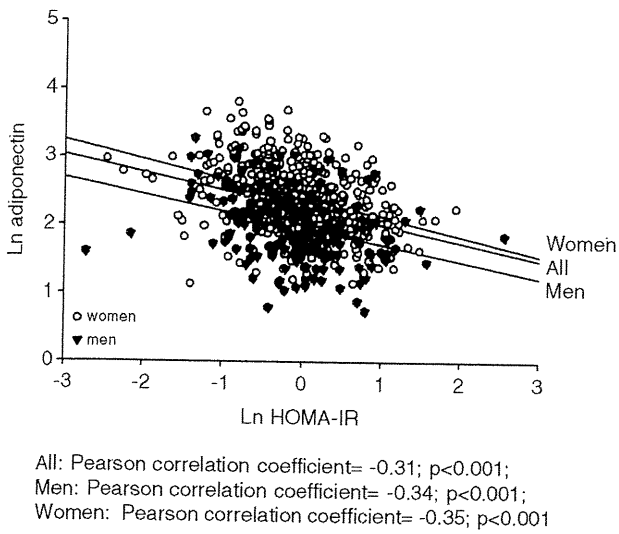
DISCUSSION

In the present study, we found that subjects with low plasma adiponectin levels had a higher incidence of cardiovascular diseases, such as heart failure and myocardial infarction, than subjects with high plasma adiponectin levels. On the other hand, we found that age, insulin resistance and the plasma levels of HDL-C, uric acid, TG and BNP were independent predictors of the plasma adiponectin level.

Table 1 Comparison of characteristics by quartiles of serum adiponectin levels in all subjects

	First quartile (n = 159)	*Second quartile (n = 159)*	*Third quartile (n = 160)*	*Fourth quartile (n = 159)*	*P-value*
Age (years)	64.0 ± 9.3	64.5 ± 9.2	66.1 ± 7.2	67.4 ± 7.1[c,e]	0.001
BMI ($kg\,m^{-2}$)	23.4 ± 2.6	23.6 ± 3.1	22.5 ± 2.8[b,d]	21.4 ± 3.1[c,e,f]	<0.0001
Waist circumference (cm)	85.6 ± 6.9	86.2 ± 8.8	83.7 ± 8.9	79.3 ± 9.8[c,e,f]	<0.0001
HbA1c (%)	5.3 ± 0.4	5.4 ± 0.6	5.3 ± 0.3	5.3 ± 0.4	0.43
HDL-C ($mg\,dl^{-1}$)	55.1 ± 13.6	58.8 ± 14.0	60.5 ± 14.1[b]	68.3 ± 15.7[c,e,f]	<0.0001
Uric acid ($mg\,dl^{-1}$)	5.8 ± 1.4	5.1 ± 1.2[a]	4.6 ± 1.2[b,d]	4.5 ± 1.1[c,e]	<0.0001
Ln TG ($mg\,dl^{-1}$)	4.8 ± 0.5	4.6 ± 0.4[a]	4.6 ± 0.5[b]	4.3 ± 0.4[c,e,f]	<0.0001
Ln adiponectin ($ng\,ml^{-1}$)	1.6 ± 0.2	2.1 ± 0.1[a]	2.5 ± 0.1[b,d]	3.0 ± 0.2[c,e,f]	<0.0001
Δ insulin ($\mu U\,ml^{-1}$)	50.7 ± 39.8	58.7 ± 53.6	40.1 ± 30.3[d]	30.9 ± 23.8[c,e]	<0.0001
Δ BG ($mg\,dl^{-1}$)	48.9 ± 43.7	47.5 ± 44.1	36.0 ± 36.8[b]	33.4 ± 45.1[c,e]	0.001
Fasting glucose ($mg\,dl^{-1}$)	97.1 ± 11.4	96.2 ± 10.4	92.8 ± 8.2[b,d]	93.1 ± [c,e]	<0.0001
Fasting insulin ($\mu U\,ml^{-1}$)	5.8 ± 4.6	5.7 ± 4.1	4.4 ± 2.3[b,d]	3.6 ± 1.9[c,e]	<0.0001
Ln HOMA-IR	0.13 ± 0.66	0.11 ± 0.64	−0.11 ± 0.55[b,d]	−0.35 ± 0.60[c,e,f]	<0.0001
Ln BNP ($pg\,ml^{-1}$)	2.7 ± 0.7	2.9 ± 0.8	3.1 ± 0.7[b]	3.4 ± 0.8[c,e,f]	<0.0001
Gender (men)	95 (59.7%)	55 (34.6%)[a]	36 (22.5%)[b,d]	19 (11.9%)[c,e,f]	<0.0001
Diagnosed DM	25 (15.7%)	14 (8.8%)	10 (6.3%)[b]	12 (7.5%)[c]	0.02

Abbreviations: BG, blood glucose; BMI, body mass index; BNP, brain natriuretic peptide; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; TG, triglyceride.
Adiponectin: First quartile, <6.66; second quartile, ⩾6.66 and <9.80; third quartile, ⩾9.80 and <14.28; fourth quartile: ⩾14.28.
[a]First *vs.* second quartile.
[b]First *vs.* third quartile.
[c]First *vs.* fourth quartile.
[d]Second *vs.* third quartile.
[e]Second *vs.* fourth quartile.
[f]Third *vs.* fourth quartile.



All: Pearson correlation coefficient= -0.31; p<0.001;
Men: Pearson correlation coefficient= -0.34; p<0.001;
Women: Pearson correlation coefficient= -0.35; p<0.001

Figure 4 Scatter plot of the association between natural log-transformed plasma adiponectin levels and homeostasis model assessment-insulin resistance (HOMA-IR) in healthy subjects. Lines indicate the regression lines.

Furthermore, we showed that the glucose spike during the OGTT was not related to the plasma adiponectin level.

What is the role of adiponectin in cardiovascular and cerebrovascular events? First, a low adiponectin level was not associated with cerebral hemorrhage or infarction after adjusting for other cardiovascular risk factors in the JMS cohort study that was performed in 12 rural districts in Japan,[12] and that the finding corresponds with the present result that the plasma adiponectin level is not related to cerebrovascular events. Second, Persson *et al.*[7] reported that low plasma adiponectin levels are associated with myocardial infarction in individuals below the age of 60 years, and Nakamura *et al.*[10] found that hypoadiponectinemia may increase the risk of acute coronary syndrome. Furthermore, in a study of Japanese men with CAD, patients with acute coronary syndrome had lower adiponectin levels than stable CAD patients, whereas patients with multiple complex lesions had significantly lower adiponectin levels than those with solitary complex lesions.[13] Thus, low adiponectin levels may be related to vulnerability to coronary atherosclerosis, which was also confirmed by the results of the present study. An important new finding of the present study is that adiponectin levels were monitored in a cohort and cardiovascular events were assessed prospectively. In our study, the low adiponectin level predicted cardiovascular events but not cerebrovascular events. The vascular or endothelial response to adiponectin may differ between the coronary and cerebral artery; however, the precise molecular mechanism underlying this difference remained unclear. Further basic and epidemiological studies are needed to elucidate the lack of a protective effect of adiponectin against stroke.

With regard to the vulnerability of coronary plaques, hypertension is believed to be one of the most important factors. Iwashima *et al.*[14] reported that the plasma adiponectin levels were lower in hypertensive subjects in a hospital-based study. In addition, two prospective Asian studies have shown that hypoadiponectinemia is associated with incident hypertension. Chow *et al.*[15] conducted a 5-year prospective study to examine the association between adiponectin and hypertension in a nondiabetic Chinese cohort. From health insurance data, 391 healthy Japanese men were followed for a period of 6 years in another study, during which 45 of them developed hypertension.[16] Our results were partially consistent with those of these studies, because four men in the low-adiponectin group developed hypertension, whereas none of the subjects in the high-adiponectin group developed hypertension. However, BP was not linked to the adiponectin levels, although in the present study we did

Table 2 Multivariate linear regression analysis of independent determinants of Ln adiponectin

	All (n = 637)		*Men (n = 205)*		*Women (n = 432)*	
	β	*P-value*	β	*P-value*	β	*P-value*
Age (years)	0.160	<0.001	0.153	0.03	0.194	<0.001
BMI ($kg\,m^{-2}$)	0.018	0.75	0.007	0.96	0.034	0.63
Waist circumference (cm)	−0.092	0.11	−0.134	0.30	−0.093	0.19
HbA1c (%)	−0.015	0.66	0.088	0.21	−0.067	0.12
HDL-C ($mg\,dl^{-1}$)	0.104	0.005	0.100	0.16	0.129	0.007
Uric acid ($mg\,dl^{-1}$)	−0.130	0.001	−0.126	0.06	−0.119	0.006
Ln TG ($mg\,dl^{-1}$)	−0.169	<0.001	−0.151	0.05	−0.179	<0.001
Δ BG ($mg\,dl^{-1}$)	0.014	0.77	−0.118	0.27	0.068	0.25
Δ insulin ($\mu U\,ml^{-1}$)	−0.062	0.12	0.127	0.10	−0.152	0.004
Ln HOMA-IR	−0.140	0.001	−0.208	0.009	−0.131	0.02
Ln BNP ($pg\,ml^{-1}$)	0.151	<0.001	0.149	0.03	0.161	<0.001
Diagnosed DM	−0.052	0.25	−0.001	0.99	−0.067	0.22
Gender (men)	−0.267	<0.001				

Abbreviations: BG, blood glucose; BMI, body mass index; BNP, brain natriuretic peptide; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; TG, triglyceride.
All: adjusted R^2:0.38; Men: adjusted R^2:0.26; Women: adjusted R^2:0.30; β: standardized regression coefficient.

not have information about the anti-hypertensive drugs used. Indeed, in the Copenhagen City Heart Study, the plasma adiponectin level was not an independent predictor of new-onset hypertension,[17] so there may be a racial difference with regard to the influence of adiponectin on hypertension. The linkage between hypertension and the plasma adiponectin level is not conclusive in the present study.

The next issue is the mechanisms by which a low adiponectin level can be linked to cardiovascular disease. Ouchi *et al.*[18] reported that hypoadiponectinemia is associated with impaired endothelium-dependent vasorelaxation, and endothelial dysfunction is an important feature of the early stages of atherosclerosis and hypertension.[19] In Apo E-deficient mice, an increase in the plasma adiponectin level suppressed the progression of atherosclerotic lesions by attenuating endothelial inflammation and transformation of macrophages to foam cells *in vivo*.[20,21] Furthermore, a low adiponectin level and/or impaired endothelial dysfunction are associated with the onset of pressure-overload heart failure.[22] whereas impaired endothelial dysfunction is related to hypertension and diastolic dysfunction,[23] which may explain the high incidence of acute heart failure in patients who have hypertension as well as those with acute coronary syndrome. Therefore, a low plasma adiponectin level may provoke coronary and myocardial changes that culminate in cardiovascular disease.

Because a low adiponectin level was associated with an increased incidence of cardiovascular disease, we investigated the determinants of adiponectin among participants who underwent the OGTT, because we hypothesized that the glucose spike during this test would be related to the plasma adiponectin level. We found that the age, gender, insulin resistance and plasma levels of TG, HDL-C, BNP and uric acid influenced the adiponectin level in all participants, which agreed with previous reports.[5,24,25] However, we found that the glucose spike during the OGTT did not affect the plasma adiponectin level, although HOMR-IR (which represents insulin resistance) had an influence on adiponectin. The present results suggest that adiponectin may have a regulatory role in insulin secretion. However, this relationship between insulin secretion and the plasma adiponectin level was found in women, but not in men, in the present study. Therefore, the plasma adiponectin level may be influenced by the pattern of abnormal glucose metabolism, with the most important factor being the level of insulin resistance rather than the glucose spike or the amount of insulin secretion. Thus, impaired signal transduction following insulin receptor activation may be linked to the plasma adiponectin level.

Overall, the results of our study were consistent with those of other population-based studies. The plasma adiponectin level was inversely associated with insulin resistance, plasma TG and plasma uric acid, whereas it was positively correlated with HDL-C and BNP levels.[4,5,24,26] The adiponectin levels increase in patients with myocardial dysfunction in our study, although adiponectin is protective against heart failure because adiponectin attenuates cardiac hypertrophy.[22,27] The plasma adiponectin level may be upregulated to compensate for cardiovascular damage because BNP can modulate adiponectin signaling via a cyclic guanosine 5′-monophosphate-mediated pathway in human adipocytes.[28] That may be one of the explanations for the positive correlation between the plasma adiponectin and BNP.

However, several limitations of the present study should be considered. The study population was only of moderate size and the percentage of men was relatively low. This cohort study focused on rural residents, who may differ in various ways from urban residents. Moreover, the follow-up period was relatively short. Nevertheless, we demonstrated that the plasma adiponectin level is tightly related to cardiovascular events, suggesting that adiponectin is a potential biomarker for predicting cardiovascular events, although further studies are required for confirmation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ORIGINAL ARTICLE
Arrhythmia/Electrophysiology

Landiolol, an Ultra-Short-Acting β_1-Blocker, More Effectively Terminates Atrial Fibrillation Than Diltiazem After Open Heart Surgery

– Prospective, Multicenter, Randomized, Open-Label Study (JL-KNIGHT Study) –

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Background: Recent studies have suggested that esmolol is the first choice for rate control in patients with postoperative atrial fibrillation (AF) after coronary artery bypass surgery, but side-effects of esmolol such as hypotension are problematic. To overcome this problem, landiolol, an ultra-short-acting β_1-blocker with a less negative inotropic effect than esmolol, has been developed. The aim of the present study was to investigate whether landiolol was effective for both rate control and conversion to normal sinus rhythm (NSR).

Methods and Results: A prospective, randomized, open-label comparison between i.v. landiolol and diltiazem in patients with postoperative AF was undertaken between January 2008 and June 2009 in Japan. Of 335 patients included in the analysis, 71 patients went into AF. Among these 71 patients, conversion to NSR within 8 h after onset of AF occurred in 19 of 35 patients (54.3%) in the landiolol group vs. 11 of 36 patients (30.6%) in the diltiazem group ($P<0.05$). The incidence of hypotension was lower in the landiolol group (4/35, 11.4%) compared with the diltiazem group (11/36, 30.6%; $P<0.05$). The incidence of bradycardia was also lower in the landiolol group (0%) compared with the diltiazem group (4/36, 11.1%; $P<0.05$).

Conclusions: Landiolol is more effective and safer than diltiazem for patients with postoperative AF after open heart surgery. (*Circ J* 2012; **76:** 1097–1101)

Key Words: Atrial fibrillation; Beta-blocker; Cardiovascular surgery; Landiolol

Supraventricular tachyarrhythmias, especially atrial fibrillation (AF), are common after open heart surgery.[1–3] In many cases, postoperative AF is transient and spontaneously reverts to sinus rhythm, but tachycardia due to prolonged AF may impair left ventricular function and cause congestive heart failure.[4] Previous studies reported that postoperative AF increases the incidence of postoperative pneumonia, myocardial infarction, and/or heart failure, followed by an increase in mortality.[5] It is also associated with increased low cardiac output syndrome and costs. Thus, heart rate and/or rhythm control are necessary to prevent cardiovascular events in patients with postoperative AF. Common therapeutic approaches to achieve rate and/or rhythm control include digoxin, β-blockers, calcium antagonists, and pharmacological or electrical cardioversion.[6] Recent studies suggest that, among these modalities, esmolol is the first-choice drug for patients with postoperative AF after coronary artery bypass grafting (CABG), but side-effects of esmolol such as hypotension are a concern.[7,8] To overcome such problems, an ultra-short-acting β_1-blocker, landiolol, has been developed by Ono Pharmaceutical in Japan. At low doses, landiolol can exert a clinically relevant negative chronotropic action without any negative inotropic effects, so that it is less

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likely to have negative inotropic effects compared with esmolol.[9,10] Although there have been several studies that support the efficacy of landiolol,[11–13] a large-scale multicenter randomized clinical trial has not been performed to assess the efficacy of landiolol for patients with postoperative AF after open heart surgery.

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The aim of the present prospective, randomized, open-label trial in patients with AF or atrial flutter (AFl) after open heart surgery was therefore to compare the efficacy and safety of landiolol to diltiazem for postoperative AF or AFl.

Methods

Study Design and Subjects

A prospective, multicenter, randomized, open-label study of i.v. landiolol vs. i.v. diltiazem in patients with postoperative AF or AFl (Japan Landiolol kick-off of novel investigation for gold standard heart study [JL-KNIGHT study]) was undertaken at 36 hospitals in Japan between January 2008 and June 2009. Patients between 20 and 85 years of age who were undergoing elective open heart surgery were eligible for inclusion. The steering committee initially planned to enroll 400 patients in the study, based on the following considerations. If the frequency of conversion to sinus rhythm after 8 h of treatment with landiolol or diltiazem is 60% and 25%, respectively, the number of subjects required would be 30 for each group based on the Mooss et al data.[8] Considering dropout and withdrawal during the study, 40 patients would be needed for each group. Consequently, we estimated that the total number of subjects should be 400, assuming that the frequency of AF after open heart surgery is 20%. Exclusion criteria were acute myocardial infarction within 3 days; history of supraventricular arrhythmia that had required treatment; sinus node disease; permanent pacemaker; severe heart failure (New York Heart Association III/IV or ejection fraction <35%); atrioventricular block (AV block; ≥second degree); contraindications to either β-blocker or calcium channel blocker therapy; AF with a known secondary cause (eg, electrolyte imbalance, Wolff-Parkinson-White syndrome, or hyperthyroidism); hypotension (<90/60 mmHg); and perioperative use of an anti-arrhythmic agent other than digitalis.

All patients provided written informed consent to this study after admission to hospital. The study protocol was approved by the institutional review boards and ethics committees of all participating hospitals, and the study was performed in accordance with the Declaration of Helsinki.

Study Protocol

When patients were enrolled in this study, one of the 2 study drugs was randomly assigned at the participating institution using the envelope method. Patients were continuously monitored on telemetry for up to 1 week after surgery. A study drug was given if postoperative AF/AFl occurred with a ventricular rate ≥100 beats/min for 5 min. Landiolol was given as a continuous infusion at an initial rate of 0.5–2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ that was titrated to a maximum rate of 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (reassessed every 10 min) based on hemodynamic or electrocardiographic responses. Diltiazem was given as a bolus dose of 0.25 mg/kg over 2 min, followed by an initial rate of 3 mg/h that was titrated to a maximum rate of 15 mg/h (reassessed at approximately 1-h intervals) based on hemodynamic or electrocardiographic responses at the investigator's discretion. The maintenance infusion of either drug was titrated upward to control the ventricular rate at <90 beats/min. Patients all received the study drugs following the onset of AF/AFl and had a treatment duration of 24 h. If hypotension (systolic blood pressure <90 mmHg) or bradycardia (heart rate <50 beats/min) occurred, the dose was down-titrated or infusion of the drug was discontinued until symptoms resolved.

The primary endpoint was frequency of conversion to sinus rhythm after 8 h of treatment. The secondary endpoints were (1) frequency of conversion to sinus rhythm after 24 h of treatment and (2) achievement of rate control (<90 beats/min).

Accordingly, the total number of treated patients who were converted to sinus rhythm or who achieved the target heart rate after 8 h and 24 h of study drug infusion was determined, and blood pressure and heart rate data were collected every 1 h following initiation of therapy. Patients were assessed for adverse reactions including AV block, bronchospasm, asystole, bradycardia, and hypotension. When AF/AFl was converted to sinus rhythm, the patient was monitored on telemetry for 3 days after the discontinuation of the drug to detect the recurrence of arrhythmia.

Statistical Analysis

Continuous variables are expressed as mean±SD. Clinical characteristics of the 2 groups were compared using an independent t-test for continuous variables and with either Fisher's exact test or a chi-square test for categorical variables. Differences of conversion rates, the percentage of patients achieving rate control, and adverse events were analyzed with Fisher's exact test or the chi-square test as appropriate. In all analyses, $P<0.05$ was considered statistically significant.

Results

A total of 420 patients from 36 hospitals participated in the study. Among them, 82 patients (19.5%) developed postoperative AF and received one of the study drugs. In all patients, AFl was not observed perioperatively. Eighty-five patients were excluded because of missing data or lack of adherence to the study protocol, therefore 335 patients were included in the final analysis. Among them, 71 patients (21.2%) developed postoperative AF and received either landiolol (n=35) or diltiazem (n=36; **Figure**). The clinical characteristics of the 2 study groups were similar (**Table 1**).

A higher conversion rate to sinus rhythm after 8 h of treatment was obtained in the landiolol group (54.3%) than the diltiazem group (30.6%; $P<0.05$; **Table 2**). In contrast, there was no difference between the landiolol and diltiazem groups with regard to conversion to sinus rhythm after 24 h (74.3% vs. 61.1%, P=NS). When the efficacy of heart rate control (<90 beats/min) was analyzed, there was no difference between the 2 groups after 8 h and 24 h of treatment. Only one of 35 patients in the landiolol group, however, had failure of heart rate control within 72 h of starting treatment, while 8 of 36 patients in the diltiazem group did not achieve heart rate control ($P<0.05$).

With respect to side-effects, the incidence of hypotension (systolic blood pressure <90 mmHg) was significantly lower in the landiolol group (11.4%; $P<0.05$) compared with the diltiazem group (30.6%; **Table 3**). In addition, 4 patients receiving diltiazem experienced bradycardia that required treatment, vs. no patients from the landiolol group ($P<0.05$). The length of intensive care unit stay, however, did not differ between the 2 groups (landiolol, 3.9±2.1 days; diltiazem, 3.4±1.2 days; NS).

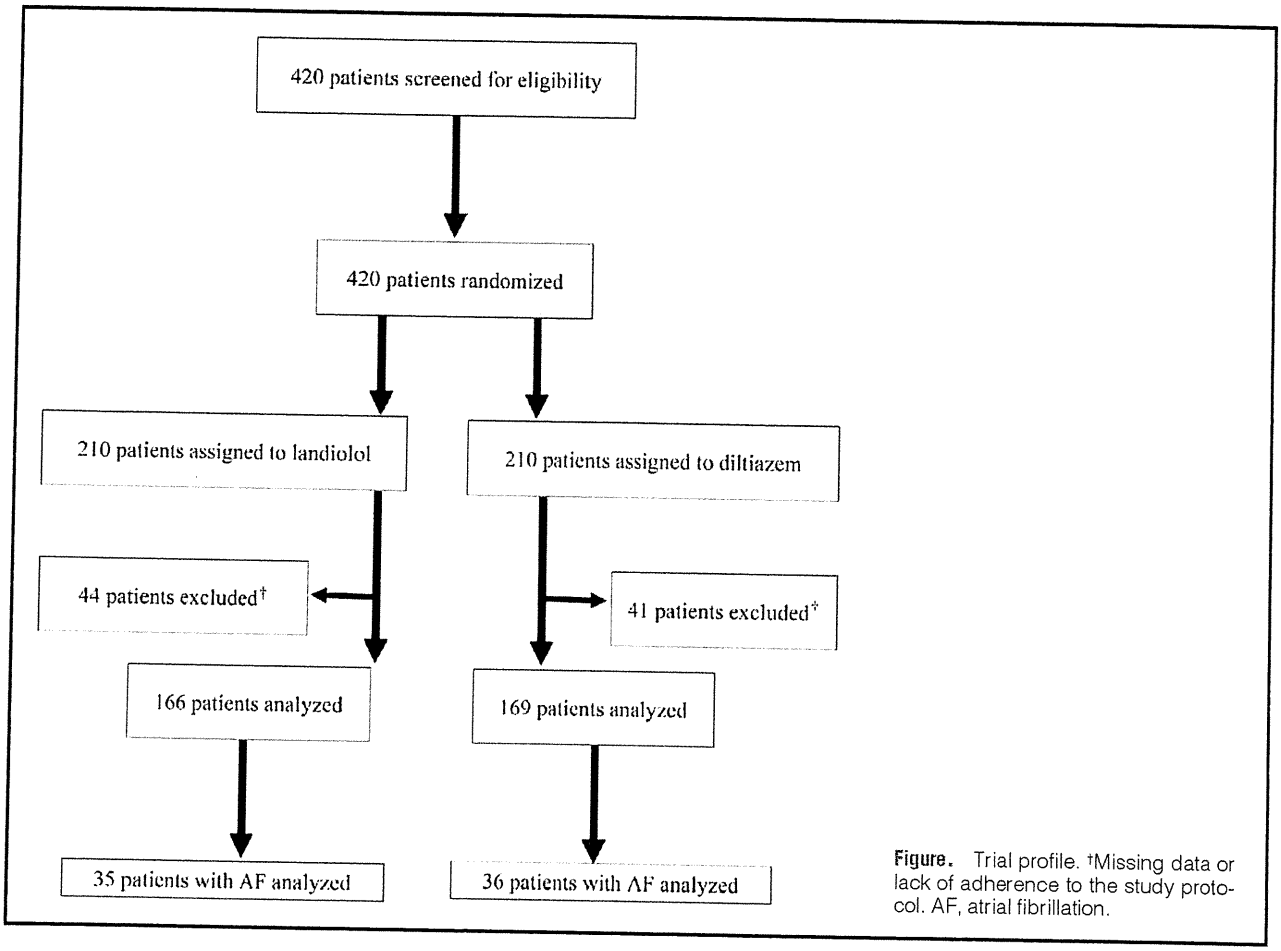

Figure. Trial profile. †Missing data or lack of adherence to the study protocol. AF, atrial fibrillation.

Table 1. Clinical Characteristics: Treated Patients

	Landiolol (n=35)	Diltiazem (n=36)
Age (years)	70.2±10.6	69.3±8.4
Sex (F/M)	11/24	12/24
Complications		
Hypertension	22	22
Diabetes	10	9
Preoperative oral β-blocker use	6	8
Surgery		
CABG	17	8
VR	10	15
CABG+VR	3	6
Others	5	7
Pre-infusion HR (beats/min)	129±20	131±20
Pre-infusion BP (mmHg)	116±26/62±14	112±17/62±13

Data given as mean±SD or n.
CABG, coronary artery bypass grafting; VR, valve replacement; HR, heart rate; BP, blood pressure.

Table 2. Treatment Effects

	Landiolol (n=35)	Diltiazem (n=36)
Conversion to sinus rhythm		
<8h	19 (54.3)*	11 (30.6)
<16h	21 (60.0)	17 (47.2)
<24h	26 (74.3)	22 (61.1)
HR controlled, but not converted		
<8h	22 (62.9)	18 (50.0)
<24h	29 (82.9)	27 (75.0)
<72h	34 (97.1)*	28 (77.8)
Recurrence of AF	3/26 (11.5)	6/22 (27.2)

Data given as n (%). *P<0.05 vs. diltiazem (X^2-test).
HR, heart rate; AF, atrial fibrillation.

Table 3. Adverse Effects and Duration of ICU Stay

	Landiolol (n=35)	Diltiazem (n=36)
Hypotension	4 (11.4)*	11 (30.6)
Bradycardia	0 (0)*	4 (11.1)
Ischemic ECG	0 (0)	2 (5.6)
AV block	0 (0)	0 (0)
Bronchospasm	0 (0)	0 (0)
ICU stay (days)	3.9±2.1	3.4±1.2

Data given as mean±SD or n (%). *P<0.05 vs. diltiazem (X^2-test).
ICU, intensive care unit; ECG, electrocardiogram; AV, atrioventricular.

Discussion

There were 2 main findings of this study. First, landiolol was more effective compared with diltiazem for conversion of AF to sinus rhythm within 8h of starting treatment. Second, landiolol treatment for postoperative AF was associated with a lower incidence of side-effects such as hypotension or bra-

dycardia compared with diltiazem.

Although it is still not clear whether the same mechanisms leading to AF in the general population are responsible for the onset of postoperative AF, current evidence suggests that its pathogenesis is multifactorial. The inflammatory response and oxidative stress associated with cardiopulmonary bypass, cardiotomy, and ischemia-reperfusion injury can induce further myocardial damage and are arrhythmogenic, which may lead to the onset of AF in patients with a susceptible anatomical substrate. Moreover, excessive sympathetic activity or suppression of parasympathetic activity can promote the onset and persistence of AF.[14] It is well known that sympathetic hyperactivity and high circulating catecholamine levels occur after cardiac surgery. Increased sympathetic activation provokes ectopic impulses, inflammation, increased vascular permeability, and altered atrial refractoriness, contributing to the creation of an arrhythmogenic substrate.[15] In the present study, landiolol was more effective for achieving conversion to sinus rhythm than diltiazem, which suggests that controlling excessive sympathetic activity may be important in the treatment of postoperative AF.

The present results for landiolol seem to be similar to those for esmolol at first assessment, but are not. Regarding the effect of esmolol vs. calcium antagonists (verapamil or diltiazem) for postoperative AF: (1) esmolol was superior to calcium antagonists in the conversion rate[8,16] and did not inhibit spontaneous conversion;[17] (2) there was no difference of heart rate control or the incidence of side-effects between esmolol and calcium antagonists;[7,8] and (3) intraoperative use of esmolol for heart rate control may worsen cardiac performance, as indicated by an increase of pulmonary arterial pressure or pulmonary vascular resistance.[18] In contrast, the present study showed that landiolol treatment led to a significantly higher rate of conversion than diltiazem treatment during the initial 8 h of therapy and that only one of 35 patients in the landiolol group had inadequate heart rate control during the 72-h observation period following the start of treatment. Landiolol, however, caused a lower incidence of side-effects such as bradycardia and hypotension compared with diltiazem, so these results differed from the data obtained for esmolol and calcium antagonists.[7,8] One explanation for the disparity between esmolol and landiolol is that landiolol is a potent short-acting and more β_1-selective blocker than esmolol. Another is that landiolol exerts a clinically relevant negative chronotropic action without any associated negative inotropic action, thus having fewer negative inotropic side-effects compared with esmolol.[9,10] Therefore, landiolol may be superior to esmolol with respect to the suppression of cardiovascular events, although we did not compare these drugs in the present study. We used low-dose infusion of landiolol at a similar level as in other studies to minimize the incidence of side-effects. Fujiwara et al reported that low-dose infusion (1.5–2.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) of landiolol for 2 days after CABG is effective and sufficient for preventing AF without suppressing cardiac function.[13] Sezai et al reported that infusion of landiolol at 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ from the time of central anastomosis during CABG and for 2 days subsequently was more effective for preventing AF than placebo and was associated with a lower incidence of major complications.[19] The incidence of side-effect in these studies was similar to that in the present study.

In the present study, infusion of the ultra-short-acting β_1-blocker landiolol ($t_{1/2}$=4 min) for only 24 h maintained heart rate control for 48 h after the cessation of treatment, and 24-h infusion of landiolol had a similar suppressant effect to diltiazem on the recurrence of AF. It is possible that control of sympathetic activity at the time close to the onset of AF may be important for treating tachycardiac AF, but the mechanism involved is still unclear. Also, the optimum timing and duration of landiolol infusion need to be determined in the future.

In addition to treatment of AF, it is also important to prevent postoperative AF.[6] Several drugs, such as amiodarone and sotalol, have shown efficacy in preventing postoperative AF, but these agents need to be started several days before surgery and have the potential to cause significant adverse effects that include hypotension, bradycardia, and severe ventricular arrhythmias. Because landiolol may be able to prevent the postoperative onset of AF, we plan to start the JL-KNIGHT study II to test the preventive effects of landiolol.

Conclusion

This study showed that landiolol is more effective and safer than diltiazem for patients with postoperative AF after open heart surgery.

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Original article

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

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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

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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 $[^3H]$ 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 $[^3H]$ 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

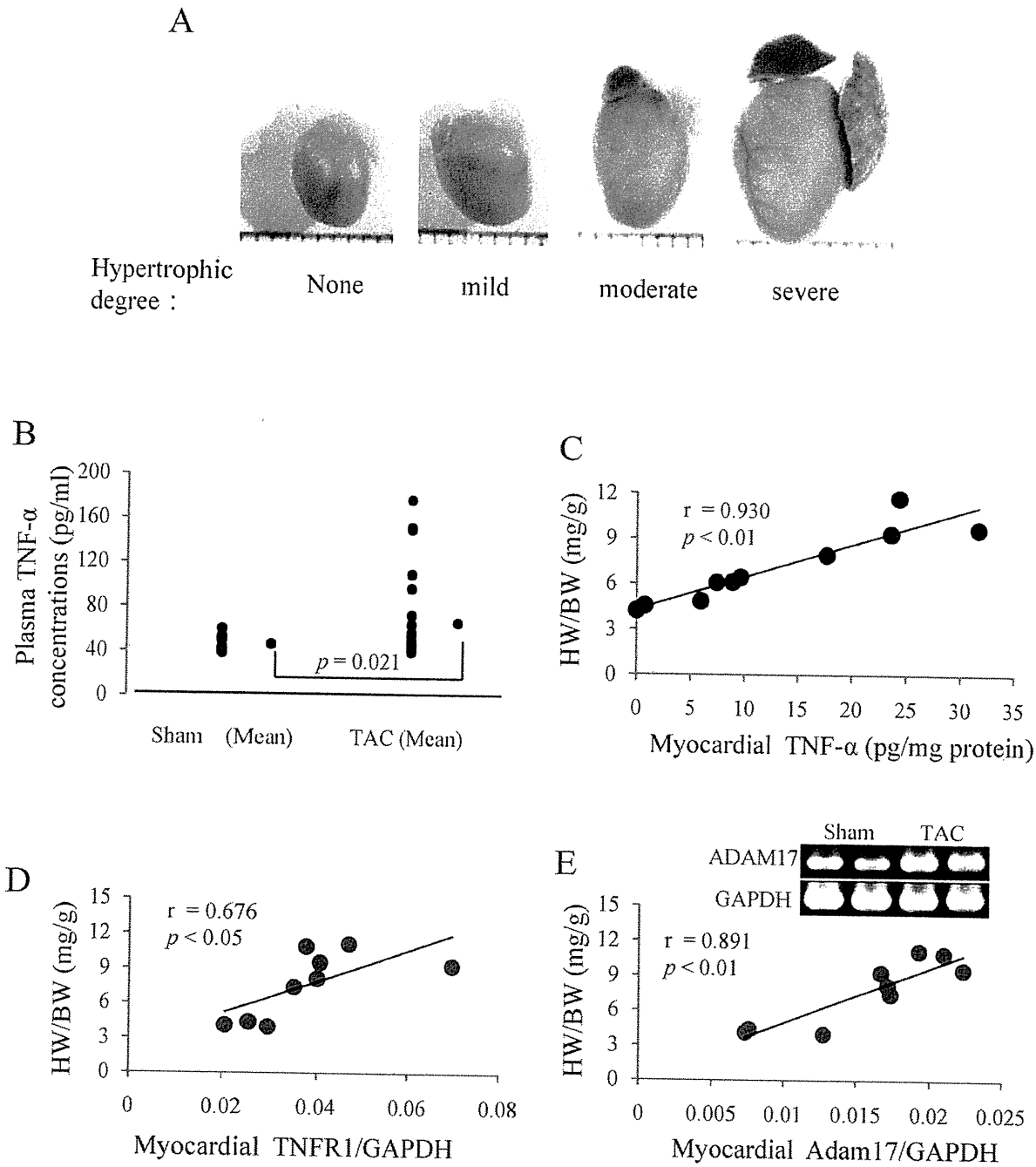

Fig. 1. Correlations between TNF-α signal molecules and cardiac hypertrophy. A. Representative pictures of whole hearts with various degree of hypertrophy. B. At 4 weeks after surgery, TNF-α concentrations were significantly higher in the TAC group ($n = 25$) than in sham group ($n = 9$). C. Correlation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

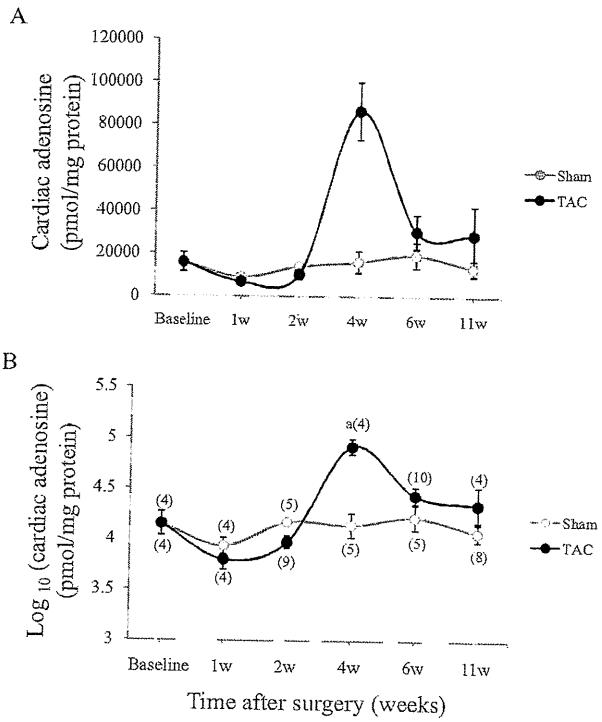

Fig. 2. Temporally changes of myocardial adenosine during cardiac hypertrophy development. Data in the panel A were transformed to logarithmic data as shown in the panel B. In TAC models, the cardiac adenosine level had a transient increase at 4 week after surgery ($^{a}P < 0.01$ vs the corresponding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

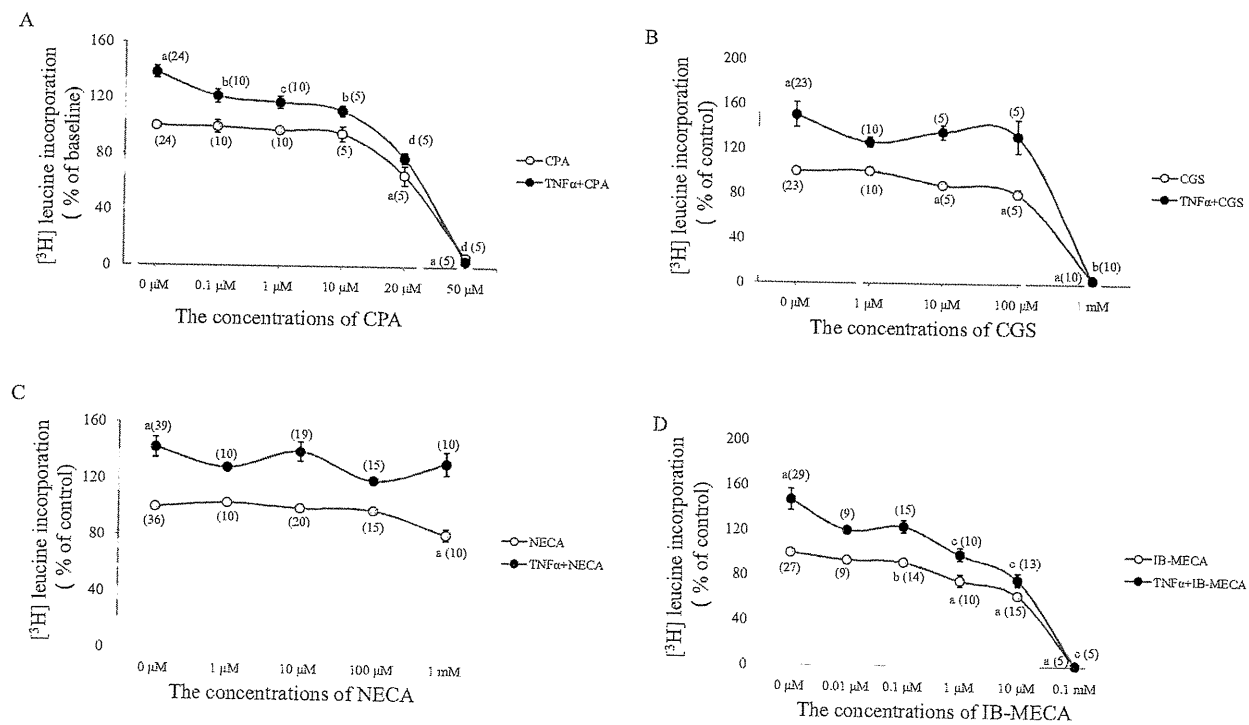

Fig. 3. Effect of adenosine agonists on TNF-α induced protein synthesis of cardiomyocytes. Incorporation of [^{3}H] leucine without addition of any agents (adenosine agonist 0 μM) was served as baseline. A. Safety range of CPA < 20 μM. $^{a}P < 0.001$ vs baseline; $^{b}P < 0.05$, $^{c}P < 0.01$ and $^{d}P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

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

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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.

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