Original Article

Usefulness of the α_1-Blocker Doxazosin as a Third-Line Antihypertensive Drug

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It has been reported that a substantial majority of hypertensives receive insufficient blood pressure (BP) control. As combination therapy for the treatment of hypertension, Ca channel blockers (CCBs), angiotensin II (AII) receptor blockers (ARBs), and/or AII-converting enzyme (ACE) inhibitors are mainly prescribed, while the efficacy of α_1-blockers in such combination therapy remains unknown. The aim of this study was to investigate the efficacy of a low dose of an α_1-blocker added to combination therapy with CCBs and either ARBs or ACE inhibitors for the treatment of hypertension. Subjects were 41 hypertensive patients (23 women and 18 men, mean age 66±12 years) who had been followed at the National Kyushu Medical Center. All patients showed poor BP control despite haven taken a combination of CCBs and ARBs or ACE inhibitors for more than 3 months. Doxazosin at a dose of 1 to 2 mg was added to each treatment regimen. The changes in various clinical parameters, including BP and blood chemistry, following the addition of doxazosin were then evaluated. The mean follow-up period was 170 days. BP decreased from 152±14/81±12 mmHg to 135±14/70±11 mmHg after the addition of doxazosin at a mean dose of 1.5 mg/day ($p<0.001$). When good systolic blood pressure (SBP) control was defined as <140 mmHg, the prevalence of patients with good SBP control increased from 24% to 61% ($p<0.01$). Similarly, the prevalence of patients with good diastolic blood pressure (DBP) control (<90 mmHg) increased from 78% to 98% ($p<0.01$). Patients whose SBP decreased more than 10 mmHg ($n=25$) showed significantly higher baseline SBP, serum total cholesterol and low-density lipoprotein (LDL) cholesterol levels compared to those who showed less SBP reduction (<10 mmHg) ($n=16$, $p<0.01$). Comparable BP reductions were obtained between obese (body mass index [BMI] ≥25, ΔBP at 3 months: −15 ± 15/−12 ± 9 mmHg, $n=18$) and non-obese (BMI<25, ΔBP: −14 ± 19/−7 ± 8 mmHg, $n=23$) patients. The results suggest that addition of a low dose of the α_1-blocker doxazosin effectively reduces BP in patients taking CCBs and ARBs or ACE inhibitors. Thus, doxazosin seems to be useful as a third-line antihypertensive drug. (*Hypertens Res* 2007; 30: 301–306)

Key Words: **α_1-blocker, antihypertensive drug, combination therapy**

Introduction

Although the guidelines for the treatment of hypertension emphasize strict blood pressure (BP) control, it has been reported that a substantial majority of hypertensives receive insufficient BP control (*1–3*). Thus, aggressive combination therapy is recommended to achieve the target BP levels (*1–5*). Ca channel blockers (CCBs) and either angiotensin II (AII) receptor blockers (ARBs) and/or AII-converting enzyme (ACE) inhibitors are widely used as a combination therapy. As to α_1-blockers, many studies have suggested that α_1-blockers reduce coronary heart disease by lowering BP and favorably affecting serum lipid profiles (*6–9*). However, the ALLHAT trial has advised against the use of α_1-blockers as first-line antihypertensive drugs (*10*). On the other hand, α_1-

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blockers have been reported to be effective in combination with other antihypertensive drugs, such as CCBs, ACE inhibitors, diuretics and β-blockers (*11–16*). However, there are insufficient data regarding the efficacy of α_1-blockers as third-line antihypertensive drugs. Thus, the aim of this study was to investigate the usefulness of a low dose of the α_1-blocker doxazosin in combination therapy with CCBs and either ARBs or ACE inhibitors.

Methods

Participants were hypertensive outpatients who visited the National Kyushu Medical Center. Patients who were newly prescribed the α_1-blocker doxazosin were retrospectively investigated. Subjects consisted of 41 hypertensive outpatients (23 men and 18 women; mean age, 66±12 years) of the National Kyushu Medical Center who had taken the combination of a CCB and either an ARB or ACE inhibitor for more than 3 months, but had failed to achieve the target BP recommended by the Japanese guideline (JSH 2004) (*1*). Doxazosin at a dose of 1 to 2 mg was added to each treatment regimen; 6 patients received doxazosin once daily in the morning, 19 patients received doxazosin once daily at bedtime, and 16 patients received doxazosin twice daily. Then, the patients were followed for at least 3 months (average 170 days). The changes in various clinical parameters, including BP and blood chemistry, following the addition of doxazosin were then evaluated. Clinic BP was measured during the morning hours with subjects in a seated position by physicians using a mercury sphygmomanometer. Diabetes mellitus (DM) was defined as fasting plasma glucose ≥126 mg/dl, plasma glucose ≥200 mg/dl at any time, HbA1c ≥6.5%, or the current use of hypoglycemic agents. Hyperlipidemia was defined as serum total cholesterol ≥220 mg/dl, serum triglyceride ≥300 mg/dl at any time, or the current use of lipid-lowering drugs. Body mass index (BMI) was calculated as BMI = weight/height^2 (kg/m^2). "Good control" was defined as a systolic blood pressure (SBP) of <140 mmHg and diastolic blood pressure (DBP) of <90 mmHg. "Satisfactory control" was defined as SBP of <130 mmHg and DBP of <85 mmHg. The protocol was explained to each patient in detail, and informed consent was obtained from each patient. This study was conducted following the guidelines of the National Kyushu Medical Center.

Statistical Analysis

Values are presented as the mean±SD. The differences in the variables were compared by one-way ANOVA. A χ^2 test was also utilized when appropriate. p values less than 0.05 were considered significant.

Results

The patient characteristics are shown in Table 1. The mean age was 67±12 years, and 56% of the patients were women. The average BP decreased from 152±14/81±12 mmHg to 135±14/70±11 mmHg after the addition of doxazosin at a mean dose of 1.5 mg/day ($p<0.01$, Fig. 1). Baseline BP, ΔBP and the mean dose of doxazosin were not significantly different among the three groups, although baseline BP tended to be lower in the patients taking doxazosin at bedtime and ΔBP tended to be higher in the patients taking doxazosin twice daily (once daily in the morning, 152±11/85±11 mmHg, −13±5/−9±8 mmHg, 1.3±0.5 mg; once daily at bedtime, 144±10/75±11 mmHg, −13±17/−9±10 mmHg, 1.4±0.5 mg; twice daily, 163±12/87±11 mmHg, −23±19/−14±14 mmHg, 1.9±0.3 mg, n.s., respectively). In addition, total cholesterol and low-density lipoprotein (LDL) cholesterol levels decreased during this period (Table 1). The prevalence of patients with good SBP control (<140 mmHg) increased from 24% to 61% ($p<0.01$, Fig. 2). The prevalence of patients with satisfactory SBP control (<130 mmHg) also increased, from 0% to 32% ($p<0.01$, Fig. 2). Similarly, the prevalence of patients with good (<90 mmHg) and satisfactory (<85 mmHg) DBP control increased from 78% to 98% ($p<0.01$) and from 68% to 90% ($p<0.05$, Fig. 2).

Patients whose SBP decreased more than 10 mmHg ($n=25$) showed significantly higher baseline SBP, serum total cholesterol and LDL cholesterol levels compared to those who showed less SBP reduction (<10 mmHg) ($n=16$) ($p<0.01$, Table 2). Patients with small SBP reduction tended to have higher serum creatinine levels. In addition, patients whose DBP decreased more than 10 mmHg ($n=18$) showed significantly higher baseline DBP and LDL cholesterol levels compared to those who showed less DBP reduction (<10 mmHg) ($n=23$) ($p<0.01$ and $p<0.05$, respectively, Table 3). Patients

Table 1. Characteristics of the Patients

	Baseline	After
Number of patients	41	—
Sex (men/women)	18/23	—
Age (years)	67±12	—
Body mass index (kg/m^2)	25±4	—
SBP (mmHg)	152±14	135±14**
DBP (mmHg)	81±12	70±11**
Pulse rate (/min)	72±9	70±9
Serum creatinine (mg/dl)	0.8±0.4	0.9±0.4
Serum total cholesterol (mg/dl)	199±27	192±32**
Serum triglyceride (mg/dl)	144±82	143±108
Serum HDL cholesterol (mg/dl)	54±15	55±14
Serum LDL cholesterol (mg/dl)	116±29	107±29*
Plasma glucose (mg/dl)	119±37	112±23
Diabetes mellitus (%)	25	—
Hyperlipidemia (%)	39	—

Values are means±SD. *$p<0.05$, **$p<0.01$ *vs.* Baseline. LDL, low-density lipoprotein; HDL, high-density lipoprotein; SBP, systolic blood pressure; DBP, diasolic blood pressure.

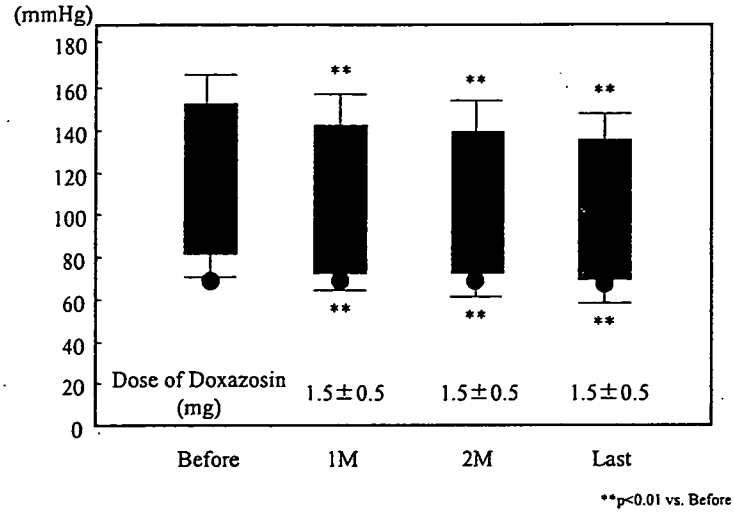

Fig. 1. *Changes in blood pressure (closed bars) and pulse rate (closed circles) by doxazosin.*

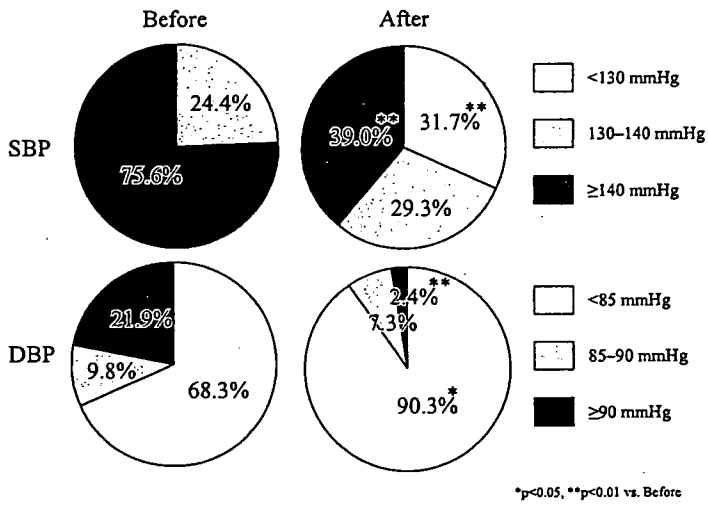

Fig. 2. *Changes in systolic blood pressure (SBP) and diastolic blood pressure (DBP) by doxazosin.*

with large DBP reduction tended to have higher BMI, and a higher dose of doxazosin was used for these patients. Comparable BP reductions were obtained between obese (BMI≥25, ΔBP: −17±18/−13±13 mmHg, n=18) and non-obese (BMI<25, ΔBP: −10±10/−3±11 mmHg, n=23) patients. In the multivariate analysis, ΔSBP was independently associated with baseline SBP. On the other hand, ΔDBP was independently associated with baseline DBP and the dose of doxazosin (Table 4).

Discussion

The results of the present study demonstrate the usefulness of a low dose of the α_1-blocker doxazosin in combination therapy.

Recent hypertension guidelines emphasize that drugs with an additive or synergistic hypotensive effect should be added to the treatment regimen if the target BP levels are not achieved (*1–3*). Combination therapy using CCBs, ARBs, and/or ACE inhibitors is quite common in Japan, but the BP control still seems to be insufficient.

Both the guidelines of the Japanese Society of Hypertension and the JNC 7 guidelines recommend the use of diuretics when more than three antihypertensive drugs are required (*1*, *2*). We have previously reported that salt intake in Japanese hypertensive patients remains high despite the increasing

Table 2. Comparison of the Characteristics between Patients with Large ΔSBP and Small ΔSBP

	ΔSBP	
	Large (≥10 mmHg)	Small (<10 mmHg)
Number of patients	25	16
Sex (men/women)	11/14	7/9
Age (years)	66±10	65±14
Body mass index (kg/m^2)	26±4	24±4
Baseline SBP (mmHg)	157±15**	145±9
Baseline DBP (mmHg)	82±12	80±13
Serum creatinine (mg/dl)	0.7±0.2#	0.9±0.5
Serum total cholesterol (mg/dl)	210±21**	180±27
Serum triglyceride (mg/dl)	146±86	142±78
Serum HDL cholesterol (mg/dl)	56±15	51±15
Serum LDL cholesterol (mg/dl)	127±24**	99±30
Serum glucose (mg/dl)	116±39	123±36
Dose of doxazosin (mg)	1.7±0.7	1.6±0.5

Values are means±SD. #$p<0.1$, **$p<0.01$ *vs.* Small. LDL, low-density lipoprotein; HDL, high-density lipoprotein; SBP, systolic blood pressure; DBP, diasolic blood pressure.

Table 3. Comparison of the Characteristics between Patients with Large ΔDBP and Small ΔDBP

	ΔDBP	
	Large (≥10 mmHg)	Small (<10 mmHg)
Number of patients	18	23
Sex (men/women)	7/11	11/12
Age (years)	67±10	65±13
Body mass index (kg/m^2)	26±5#	24±3
Baseline SBP (mmHg)	154±16	151±13
Baseline DBP (mmHg)	87±10**	77±12
Serum creatinine (mg/dl)	0.9±0.5	0.8±0.2
Serum total cholesterol (mg/dl)	203±27	195±28
Serum triglyceride (mg/dl)	112±38*	171±99
Serum HDL cholesterol (mg/dl)	52±13	55±17
Serum LDL cholesterol (mg/dl)	129±28*	105±27
Serum glucose (mg/dl)	125±47	115±29
Dose of doxazosin (mg)	1.8±0.7#	1.5±0.5

Values are means±SD. #$p<0.1$, *$p<0.05$, **$p<0.01$ *vs.* Small. LDL, low-density lipoprotein; HDL, high-density lipoprotein; SBP, systolic blood pressure; DBP, diasolic blood pressure.

emphasis on dietary modification, and that very few patients achieve the salt restriction recommended by the guidelines (*17*, *18*). Thus, the use of diuretics seems to be reasonable. However, diuretics have a number of side effects, including potassium depletion, hyperuricemia, hyperlipidemia and impaired glucose tolerance that may limit their use. Indeed, the ASCOT and ALLHAT studies have shown that diuretics, particularly when used as a combination therapy with β-blockers, may increase the risk of new-onset diabetes and may not improve the long-term prognosis (*10*, *19*).

In contrast to the disadvantages of diuretics, many studies have reported that α_1-blockers are well tolerated not only as first-line, but also as second-line drugs (*6–9*, *11–16*, *20*). In the previous studies, potent antihypertensive effects were observed when doxazosin was added to β-blockers, ACE inhibitors and/or CCBs (*11–16*). Furthermore, the low dose range of doxazosin used as monotherapy as well as combination therapy has been reported to be free of side effects (*5*). Despite the disappointing results of the ALLHAT study, which indicated that α_1-blockers increase the risk of combined cardiovascular disease events (*21*), there are some justifications for the use of α_1-blockers. In addition to their BP-lowering action, α_1-blockers have favorable effects on glucose and lipid metabolism. Shieh *et al.* have indicated that doxazosin improves glucose and insulin metabolism (*8*). Beneficial effects of doxazosin on lipid profiles have also been reported in other studies (*6–9*). Consistent with these reports, our study demonstrated that doxazosin decreased total cholesterol and LDL cholesterol levels. On the other hand, serum triglyceride and plasma glucose levels remained unchanged, although we did not evaluate insulin resistance. Thus the positive effects of doxazosin were less pronounced than expected, especially in light of another clinical trial that demonstrated a positive effect of doxazosin (*9*). In some reports, the change in triglyceride level was of lesser magnitude than that in cholesterol level (*6*, *8*). In addition, another study reported that cholesterol and triglyceride levels were significantly decreased by doxazosin monotherapy, although there was no change in the glucose level. In contrast, the cholesterol, triglyceride and glucose levels remained unchanged in patients receiving doxazosin combination therapy (*7*). Although the mechanism by which α_1-blockers effect serum lipid profiles is unclear, several hypotheses have been put forward. These include 1) stimulation of lipoprotein lipase activity, 2) reduction in very low density lipoprotein synthesis and secretion, 3) increase in LDL receptor number, and 4) decrease in cholesterol synthesis. Considering recent evidence that the management of diabetes and dyslipidemia in hypertensive patients is important for the prevention of cardiovascular diseases, the use of α_1-blockers as part of a combination therapy should be preferentially considered.

α_1-Blockers can also be used to suppress morning surge in BP. The HALT study suggested that nighttime dosing of doxazosin suppressed the BP morning surge that was associated with an increase in α_1-adrenergic activity (*22*, *23*). The peak effect of doxazosin might be determined not only by the pharmacokinetics of the drug, but also by the level of vascular tone (*21*). It is expected that an α_1-adrenergic antagonist would have its greatest effect when α_1-adrenergic tone is at its greatest. Morning surge could be a new therapeutic target for preventing target organ damage and subsequent cardiovascular events in hypertensive patients (*24*, *25*). Thus, addition of

Table 4. Clinical Factors Affecting ΔSBP and ΔDBP: Multivariate Analysis

	ΔSBP			ΔDBP		
	β	Partial r^2	p	β	Partial r^2	p
Baseline SBP (mmHg)	−0.784	0.392	<0.001	—	—	—
Baseline DBP (mmHg)	—	—	—	−0.365	0.272	<0.001
Dose of doxazosin (mg)	—	—	—	−5.090	0.120	0.013

Independent variables: age, sex, body mass index, baseline SBP/DBP, serum total cholesterol, plasma glucose. SBP, systolic blood pressure; DBP, diasolic blood pressure.

doxazosin seems to be reasonable, when the combination therapy with CCBs and ARBs or ACE inhibitors is insufficient to suppress α_1-adrenergic activity.

Finally, it is noteworthy that comparable BP reductions were obtained between obese and non-obese patients in the present study. This result might have attributable to the fact that we used a higher dose of doxazosin in the obese patients. In fact, Toyonaga *et al.* reported that doxazosin was dose-dependently effective in patients with obesity-associated hypertension (*26*). It has been suggested that the mechanism underlying the association between obesity and hypertension is the activation of the sympathetic nervous system caused by insulin resistance or hyperleptinemia. α_1-Blockers exert their antihypertensive effect by inhibiting the α_1-receptors of the sympathetic nervous system, causing relaxation of the vascular smooth muscles and lowering vascular resistance. Since α_1-blockers have a beneficial effect on insulin resistance and lipid metabolism, they may be suitable for patients with obesity-associated hypertension.

In conclusion, the addition of a low dose of doxazosin to the treatment regimen of patients taking CCBs and either ARBs or ACE inhibitors improved BP control and had beneficial effects on glucose and lipid metabolism. Thus, doxazosin seems to be useful as a third-line antihypertensive drug.

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Renal and Carotid Vascular Resistance Assessed with Doppler Sonography

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ABSTRACT: *Purpose.* Resistance index (RI) is widely used for the evaluation of circulatory resistance and atherosclerosis with Doppler sonography, but differences in RI among vascular beds have not been fully elucidated. The present study was designed to evaluate the relationship between renal and carotid artery RI and to compare their relative risk factors for an increase in RI.

Methods. One hundred eighty-five inpatients who underwent sonographic assessment of the renal and carotid arteries were enrolled in the study.

Results. Multivariate analyses revealed that age, pulse pressure (PP), and serum glucose level were positively correlated, whereas diastolic blood pressure (DBP) and creatinine clearance were negatively correlated with the RI of the interlobar arteries. Sex (male) and PP correlated positively, whereas DBP correlated negatively with the RI of the common carotid artery (CCA). The RI of the interlobar arteries was positively associated with that of the CCA, even after adjustment for major cardiovascular risk factors.

Conclusions. These findings suggest that RI of the renal and carotid arteries increase in parallel to a certain extent. On the other hand, risk factors for the increase of RI of the carotid and renal arteries differed in part, suggesting that specific control of respective risk factors may also be needed to prevent vascular damage in each vascular bed. © 2007 Wiley Periodicals, Inc. *J Clin Ultrasound* 36:85–90, 2008; Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jcu.20444

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Duplex Doppler sonography is a noninvasive method of semiquantitative evaluation of local vascular resistance, such as resistance index (RI). Measurement of renal RI is useful for the evaluation of renal parenchymal damage[1–6] and may provide a reliable marker of renal atherosclerosis.[7–10] Furthermore, RI of intrarenal arteries has been shown to be associated with the severity of target organ damage in patients with hypertension and diabetes mellitus, as well as chronic renal failure.[11–13]

RI of the common carotid artery (CCA) can be measured with Doppler sonography for the evaluation of downstream circulatory resistance. However, carotid intima–media thickness (IMT) is acknowledged as a more reliable and accurate marker of atherosclerosis, and its increase has been shown to be related to cardiovascular complications and future cardiovascular events.[14–18] On the other hand, only limited information is available concerning the relationship between carotid hemodynamics and the degree of atherosclerosis,[14,19,20] and it is unclear whether hemodynamic alterations in CCA correlate with those in other vascular beds, such as the renal artery (RA).[21] Furthermore, no study has yet addressed the differences in risk factors for the increase in vascular resistance between the RA and CCA in the same study population.

The present study was designed to elucidate the relationship between the RI of RA and

CCA and to clarify possible differences in their relateive risk factors for an increase in RI.

PATIENTS AND METHODS

Between June 2000 and February 2004, we evaluated 185 patients who were admitted to the Kyushu University Hospital for the evaluation of hypertension, diabetes mellitus, renal dysfunction, or stroke. Hypertension was defined as systolic blood pressure $\geq$140 mm Hg and/or diastolic blood pressure (DBP) $\geq$90 mm Hg or the current use of antihypertensive drugs. Diabetes mellitus was defined as fasting serum glucose $\geq$126 mg/dl or serum glucose at any time $\geq$200 mg/dl or HbA1c $\geq$6.5% or the current use of hypoglycemic agents. Renal dysfunction was assumed in patients who had increased serum creatinine levels (male $\geq$1.3 mg/dl, female $\geq$1.2 mg/dl) or persistent proteinuria or was diagnosed via histologic examination of renal specimens obtained through percutaneous renal biopsy. Stroke was diagnosed according to patient history, neurologic examination, and radiologic examination, including brain CT or MRI. Patients who had had a stroke within 3 months were excluded. In all patients, 24-hour urine collections was performed to determine creatinine clearance (Ccr). Ccr was corrected by the surface area of 1.73 m^2. Proteinuria and hematuria were defined as a dipstick reading of greater than +1. Patients who had RA or CCA stenosis, unilateral kidney, or severe renal failure (serum creatinine $\geq$6 mg/dl) were excluded from the study. Stenosis was defined as $\geq$50% stenosis in diameter using standard Doppler sonography, magnetic resonance angiography, or angiography criteria. Blood samples were drawn from patients after an overnight fasting a few days before the sonographic examination. Blood pressure was measured with a sphygmotonometer on the day of the examination with the patient in the supine position. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Informed consent was obtained from each patient. The study was conducted following the guidelines for clinical research of the ethical committee of Kyushu University Hospital.

Duplex Doppler Analysis of RA

All patients were examined in the morning after an overnight fasting. Gray-scale images of the kidney and Doppler tracing of the RA were obtained with a duplex Doppler apparatus (Powervision 6000, Toshiba, Tokyo, Japan) equipped with a convex 3.5-MHz probe. The Doppler sampling gate was placed on the main RA and on the interlobar arteries visualized with color Doppler sonography. The insonation angle was kept under 60° and precise angle correction was performed to allow for accurate blood flow velocity measurement. RI was calculated as follows: (peak systolic velocity − end diastolic velocity)/peak systolic velocity. RI was determined at least 3 times for both kidneys and was averaged to obtain the mean value of RI for each patient. All Doppler measurements were performed by the same investigator, who was blinded to the medical status of the patients. Intraobserver coefficients of variance of RI was 4.8% for the main RA and 5.2% for the interlobar arteries (n = 10).

Duplex Doppler Analysis of CCA

Sonograms of the CCA were obtained with the same ultrasound unit as for the RA with a 7.5-MHz linear probe. With the patient in the supine position, we adusted the probe position to achieve optimal visualization of the CCA. The IMT of the far wall was measured in the CCA at a point located 1–2 cm proximal to the carotid bulb. The Doppler sampling gate was placed within the CCA lumen visualized on color Doppler sonography, and the insonation was kept under 60°. Precise angle correction was performed before flow velocities were measured. RI was calculated in both CCAs and was averaged to obtain the mean value of RI for each patient. All Doppler measurements were performed by the same investigator who was blinded to the patient's medical history. Intraobserver coefficients of variance of RI of CCA was 3.0% (n = 10).

Statistical Analysis

Statistical analysis was performed with SAS software for Windows (SAS Institute, Cary, NC). The linear correlation between the variables was evaluated using Pearson and Spearman tests. Multivariate analysis (stepwise method) was also used with renal RI and carotid RI as a dependent variable with a set of independent variables (age, sex, pulse pressure [PP], DBP, serum total cholesterol, serum glucose, BMI, hematocrit, Ccr). All data are presented as the mean ± SD. A p value of less than 0.05 was considered statistically significant.

TABLE 1
Clinical Characteristics and Laboratory Results in 185 Study Patients

Men/Women	104/81
Age (years)	61.7 ± 13.7 (range, 23–87)
Systolic blood pressure (mmHg)	145 ± 20
Diastolic blood pressure (mmHg)	83 ± 14
Pulse pressure (mmHg)	63 ± 16
Serum total cholesterol (mg/dl)	197 ± 48
Serum glucose (mg/dl)	121 ± 61
Body mass index (kg/m^2)	23 ± 4
Hematocrit (%)	38 ± 7
Creatinine clearance (ml/min/1.73m^2)	73 ± 38
Main renal arteries RI	0.71 ± 0.09
Interlobar arteries RI	0.66 ± 0.09
CCA RI	0.72 ± 0.07
CCA intima-media thickness (mm)	0.83 ± 0.20
CCA max intima-media thickness (mm)	1.52 ± 0.85
Prevalence of hypertension (%)	98
Prevalence of diabetes mellitus (%)	50
Prevalence of renal dysfunction (%)	37
Prevalence of stroke (%)	50

Values are expressed as the mean ± SD.
Abbreviations: CCA, common carotid artery; RI, resistance index.

TABLE 2
Correlation between RI Values of the Main and Interlobar Renal Arteries with Clinical Factors (Univariate Analysis)

	Main Renal Arteries RI		Interlobar Arteries RI	
	r Coefficient	*p* Value	*r* Coefficient	*p* Value
Age	0.402	<0.001	0.596	<0.001
Sex	0.044	NS	−0.078	NS
Systolic blood pressure	0.147	NS	0.061	NS
Diastolic blood pressure	−0.360	<0.001	−0.448	<0.001
Pulse pressure	0.497	<0.001	0.450	<0.001
Serum total cholesterol	0.099	NS	0.142	NS
Serum glucose	0.095	NS	0.232	0.002
Body mass index	−0.021	NS	−0.042	NS
Hematocrit	−0.427	<0.001	−0.358	<0.001
Creatinine clearance	−0.415	<0.001	−0.440	<0.001
CCA RI	0.294	<0.001	0.407	<0.001

Abbreviations: CCA, common carotid artery; NS, not significant; RI, resistance index.

RESULTS

Table 1 shows the clinical characteristics of the study subjects. In univariate analyses, RI of the main renal arteries showed positive correlations with age and PP, whereas RI of the CCA showed negative correlations with DBP, hematocrit, and Ccr (Table 2). RI of the interlobar arteries showed positive correlations with age, PP, serum glucose, and CCA RI and negative correlations with DBP, hematocrit, and Ccr (Table 2). In the multivariate analysis, DBP, PP, and Ccr were independently associated with RI of the main renal arteries. Age, DBP, PP, serum glucose and Ccr were independently associated with RI of the interlobar arteries (Table 3).

Table 4 shows the associations of RI of the CCA with clinical factors in univariate analyses. RI of the CCA showed positive correlations with sex (male), PP, RA, RI, and maximum IMT of the CCA, and negative correlations with DBP. In the multivariate analysis, sex, PP, and DBP were independently associated with the RI of the CCA (Table 5).

After adjusting for age, sex, DBP, PP, total cholesterol, serum glucose, BMI, hematocrit, and Ccr, RI of the CCA was positively correlated with RI of the interlobar renal arteries, and tended to be associated with RI of the main renal arteries ($p = 0.075$). Figure 1 shows the univariate association between RIs of the main RA and interlobar arteries and RI of the CCA.

DISCUSSION

Using Duplex Doppler sonography, we found a significant correlation between RA and CCA RI. On the other hand, risk factors for the increase in vascular resistance differed in part between RA and CCA.

RI is a hemodynamic parameter that is easily determined on Doppler sonography and reflects the degree of downstream vascular resistance. Renal RI has been demonstrated to be a reliable marker of renal atherosclerosis[7–9] and renal function.[11–13]

With respect to carotid hemodynamics, Frauchiger et al[19] reported that RI of the CCA and of the internal carotid artery (ICA) was associated with atherosclerotic risk scores. Nakatou et al[14] also reported that the RI and pulsatility index of the CCA correlated significantly with atherosclerosis risk scores and the presence of brain infarction. In our study, RI of the CCA was associated with the CCA's IMT, further suggesting that RI of the CCA could also be a marker of atherosclerosis, although such an association between RI and IMT was weakened after adjustment for established cardiovascular risk factors. One of the advantages of measuring RI over IMT measurement may be that RI can be measured automatically by most ultrasound scanners, which will probably result in a lesser interobserver and intraobserver variability than that associated with IMT.[19]

In the present study, the RI of the CCA showed significant correlations with the RI of the interlobar arteries of both kidneys and tended to be correlated with the RI of the main RAs. In a previous study of 51 patients with essential hypertension, the relative diastolic flow velocity of the CCA correlated with RI of the interlobar renal

TABLE 3
Correlation between the RI Values of the Main and Interlobar Renal Arteries and Clinical Factors (Multivariate Analysis)

	Main Renal Artery RI			Interlobar Artery RI		
	β	R^2	*p* Value	β	R^2	*p* Value
Age	—	—	NS	0.002	0.361	<0.001
Diastolic blood pressure	−0.002	0.117	<0.001	−0.002	0.060	<0.001
Pulse pressure	0.002	0.247	<0.001	0.001	0.090	<0.001
Serum total cholesterol	—	—	NS	0.000	0.009	NS
Serum glucose	—	—	NS	0.000	0.023	<0.01
Hematocrit	−0.003	0.012	NS	−0.002	0.009	NS
Creatinine clearance	−0.001	0.093	<0.001	−0.000	0.043	<0.001

Adjusted value: age, sex, diastolic blood pressure, pulse pressure, serum total cholesterol, serum glucose, body mass index, hematocrit, creatinine clearance.
Abbreviations: NS, not significant; RI, resistive index.

TABLE 4
Correlation between the RI Value of the Common Carotid Artery with Clinical Factors (Univariate Analysis)

	r Coefficient	*p* Value
Age	0.107	NS
Sex	0.188	<0.05
Systolic blood pressure	0.054	NS
Diastolic blood pressure	−0.261	<0.001
Pulse pressure	0.281	<0.001
Serum total cholesterol	−0.062	NS
Serum glucose	0.073	NS
Body mass index	0.018	NS
Hematocrit	−0.081	NS
Creatinine clearance	−0.126	NS
Main renal arteries RI	0.294	<0.001
Interlobar arteries RI	0.407	<0.001
CCA maximum intima–media thickness (mm)	0.190	<0.05

Abbreviations: CCA, common carotid artery; NS, not significant; RI, resistance index.

TABLE 5
Correlation between the RI Values of the Common Carotid Artery and Clinical Factors (Multivariate Analysis)

	β	R^2	*p* Value
Sex	0.042	0.063	<0.001
Diastolic blood pressure	−0.001	0.063	<0.001
Pulse pressure	0.001	0.078	<0.001

Adjusted value: age, sex, diastolic blood pressure, pulse pressure, serum total cholesterol, serum glucose, body mass index, hematocrit, creatinine clearance.

arteries[21]; however, ours is the first study to demonstrate the direct association of RI of the CCA and RA in a large series of subjects, most of whom had hypertension. The fact that RI of CCA correlated with RI suggests that increases in vascular resistance may develop in parallel in these 2 different vascular beds. We have previously demonstrated that the renal vascular resistance was associated with pulse wave velocity.[22] It remains to be elucidated whether RI of the CCA may also be related to pulse wave velocity, a marker of systemic atherosclerosis.

Risk factors for increased vascular resistance appeared to differ in part between the RA and CCA in the present study. In multivariate analyses adjusted for major cardiovascular risk factors, RI of the main RAs was correlated with DBP, PP, and Ccr, and RI of the interlobar arteries was correlated with age, DBP, PP, serum glucose, and Ccr. On the other hand, RI of the CCA was correlated with sex (male), PP, and DBP. These results suggest that the mechanism of progressive damage and injury may in part differ between renal and carotid vascular beds. It appears that, in addition to traditional cardiovascular risk factors, renal function is strongly associated with renal vascular resistance, whereas blood pressure variables seemed to be the strongest determinant of carotid RI. Considering that carotid hemodynamic alterations are risk factors for ischemic stroke,[14] our findings may be consistent with the well-established fact that hypertension is one of the most important risk factors for stroke.[23] The mechanism of the regional differences in risk factors for an increase in vascular resistances remains incompletely understood and requires further investigation.[24]

One of the limitations of this study was that we only measured RI of the CCA which precedes 2 different circulatory beds (ie, the intracranial and extracranial arteries). In a previous study, correlation of RI of the ICA with atherosclerotic scores was stronger than that of the CCA.[19] Although the CCA is easily accessible and suitable for routine examinations, measurement of RI of the ICA should also be desirable in future studies. Another limitation of the present study was that some of the study subjects were receiving medications for hypertension, diabetes mellitus, or renal disease. Although the drug was withdrawn on the day of the examination, possible effect of drug treatment on RI remains to be

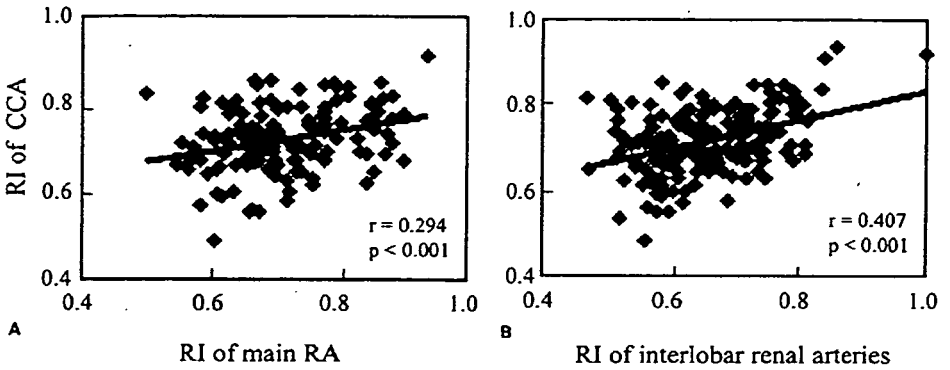

FIGURE 1. (A) Correlation between resistance index of the common carotid artery and of the main renal artery. (B) Correlation between resistance index of the common carotid artery and of the interlobar renal arteries.

determined. It also remains to be determined whether the present findings can be applied to healthy controls. Because we included only Japanese subjects, there was no need to make adjustment for ethnicity; however, it is unclear whether the present findings can be extrapolated to other ethnic populations. Finally, the number of subjects in this study may not be large enough to ensure sufficient statistical power for some variables and their correlations.

In conclusion, carotid vascular resistance was positively correlated with renal vascular resistance, indicating that an increase in vascular resistance in 2 vascular beds may develop in parallel to a certain extent. On the other hand, risk factors for an increase in vascular resistance differed in part between RAs and CCAs, suggesting that more specific control of respective risk factors may also be needed to prevent vascular damage in each vascular bed.

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Reduced-energy diet improves survival of obese KKAy mice with viral myocarditis: Induction of cardiac adiponectin expression

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Abstract

Obesity is an important risk factor for heart disease. Whether weight loss affects the severity of heart failure induced by viral myocarditis is a matter of debate. We hypothesized that weight loss could improve cardiac dysfunction by inducing cardiac expression of a cardioprotective cytokine, adiponectin. We examined the relationship between weight loss by food restriction and heart failure due to viral myocarditis in obese KKAy mice. We intraperitoneally injected encephalomyocarditis virus (500 plaque-forming units/mouse) into KKAy mice fed ad libitum as a control (CF) or 60% restriction of that eaten by ad libitum (RF). The 14-day survival rate was 0% in FF, whereas it was 23% in RF ($P<0.01$). Heart weight/body weight ratio in RF was lower than that in FF on day 5 after viral inoculation ($P<0.05$). Histological scores for myocardial necrosis and inflammation on day 5 were significantly lower in RF than in FF ($P<0.05$). Circulating adiponectin level on day 0 was significantly elevated in RF compared with that in FF (32+9 vs. 22+2 μg/mL, $P<0.05$). Comparative expression of cardiac adiponectin mRNA in RF was significantly higher than that in FF (5.1+0.3 vs. 1+0.2, $P<0.05$). Cardiac tumor necrosis factor-alpha (TNF-alpha) mRNA in RF was significantly decreased compared with that in FF on day 5 ($P<0.05$). Cardiac expression of nuclear factor kappa B was reduced and that of peroxisome proliferator-activated receptor gamma mRNA was increased in RF in comparison with FF on day 0. Cardiac adiponectin mRNA was negatively correlated with cardiac TNF-alpha mRNA ($r=-0.555$; $P=0.0097$).

Weight loss improved the survival and myocardial damage in obese mice with viral myocarditis, with cardiac induction of adiponectin. The induction of adiponectin might provide benefit through a cardioprotective effect against acute heart failure due to viral myocarditis in obese subjects.

Keywords: Obesity; Food restriction; Myocarditis; Adiponectin

1. Introduction

Obesity is an important risk factor for heart disease including congestive heart failure. Weight loss in obese patients can improve or prevent many obesity-related risk factors for heart failure through an improvement of metabolic regulation and inflammation [1,2], and can improve cardiac function [3]. Nutritional homeostasis is linked to inflammatory and immune reaction. In a recent review, a weight-management strategy is the primary therapy in obese patients [4]. Inflammatory viral myocarditis is a frequent and the cause of dilated cardiomyopathy. However, little is known about the relation between obesity and acute heart failure due to viral myocarditis.

Adiponectin is an adipocyte-specific protein which was found to be inversely correlated with obesity and cardiovascular disease [5–7]. Adiponectin also possesses anti-inflammatory

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and anti-atherogenic properties [7,8]. Thus, increasing evidence supports the notion that body-weight reduction increases circulating adiponectin, suggesting that its production is under feedback inhibition in obesity [9].

The purpose of this experiment was to show that determine whether weight loss improved survival and myocardial damage in an obese KKAy mouse model with viral myocarditis. We showed enhanced expression of peroxisome proliferator-activated receptor γ (PPARγ) and reduced expression of nuclear factor κB (NF-κB) in the myocardium of lean KKAy compared with obese KKAy mice, and that an0 adiponectin, was also upregulated in heart tissue, inversely correlated with cardiac tumor necrosis factor-α (TNF-α) mRNA level.

2. Methods

2.1. Animal model

Eight-week-old female KKAy mice were purchased from Clea (Tokyo, Japan). KKAy mice at 9 weeks of age, confirmed to exhibit hyperglycemia, were randomly divided into two groups ($n=16$ in each group): regular food ad libitum as a control (CF) and 60% restriction of that eaten by ad libitum (RF) for 2 weeks. The D variant of the encephalomyocarditis (EMC) virus (obtained from Y. Seto, Keio University, Tokyo, Japan) was stored at −70 °C in Eagle's MEM supplemented with 0.1% fetal bovine serum until use. The mice were inoculated intraperitoneally with 500 plaque-forming units of EMC virus in 0.1 ml saline. No virus was detected by assay of viral titer on day 14. The animals were placed in isolated cages and fed a diet and water. The University Committee on Animal Care of Kanazawa Medical University approved the entire experimental protocol.

2.2. Measurement of plasma adiponectin and glucose

Plasma adiponectin was measured by RIA (Linco, St. Charles, MO). Sensitivity was 1 ng/ml. Blood glucose concentration was determined by glucose oxidase method using a Fuji Dry Chem System (Medical System Co., Tokyo, Japan).

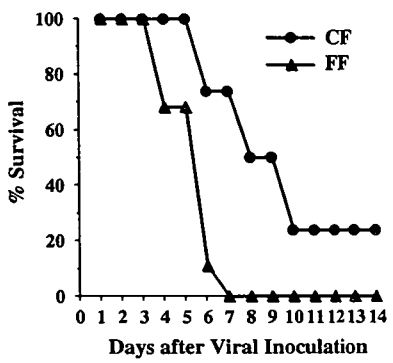

Fig. 1. Survival of KKAy mice after viral inoculation. Survival was significantly ($P<0.01$) improved in the group with 60% food restriction (RF) as compared with mice with food ad libitum as control (CF) after viral inoculation.

Table 1
Body weight and organ weight in KKAy mice after viral inoculation

	CF in KKAy mice		RF in KKAy mice	
Number	5	5	5	5
Days after inoculation	0	5	0	5
Body weight (g)	29.1+0.4	28.0+2.5	21.1+0.3*	22.8+0.2*
Thymus weight/Body weight ratio (mg/g)	2.83+0.22	1.99+0.27	2.29+0.15*	1.84+0.20
Spleen weight/body weight ratio (mg/g)	3.92+0.28	4.23+0.30	2.18+0.54*	4.25+0.46
Liver weight/body weight ratio (mg/g)	5.16+0.20	5.01+0.71	3.56+0.61*	4.66+0.93**
Blood glucose (mg/dl)	172+9	109+43	95+4*	103+22
Plasma adiponectin (μg/mL)	21.8+1.9	24.3+1.4	32.3+8.7*	27.4+0.4*

*$P<0.05$ vs. CF on the same day, CF; regular food ad libitum as a control, RF; 60% food intake.

2.3. Pathological examination

The heart and other organs were weighed. Body weight (BW) was also recorded. One half of each organ was fixed in 10% buffered formalin for tissue staining and for immunohistochemical studies; the other half was frozen for molecular study. Transverse sections of ventricular myocardium were graded for the severity of necrosis and mononuclear cell infiltration, with scoring from 1 to 4 as follows: grade 1, lesions involving <25% of the ventricular myocardium; grade 2, 25% to 50% of the myocardium; grade 3, 50% to 75% of the myocardium; and grade 4, 75% to 100% of the myocardium. The spleen, thymus, and liver were examined grossly and microscopically. Tissues were evaluated blindly by an experienced pathologist who was familiar with the grading of murine viral myocarditis and had no knowledge of the study design.

2.4. Measurement of myofiber diameter

In the lateral wall of the left ventricle, myocardial fiber diameter was determined by measuring the shortest diameter at the level of the nucleus of 50 myocardial fibers from each group with an ocular micrometer in stained cross-sections.

2.5. Immunohistochemical examination

To visualize the presence and anatomic localization of adiponectin within the myocardium, immunohistochemical studies were performed. Immunohistochemical staining was performed by the avidin biotin complex method (Vectastain ABC kit, Vector Laboratories, Burlingame, CA) previously described [10]. To minimize background staining, all sections were first blocked with normal goat serum for 20 min at room temperature. Next, the slides were incubated with an antibody directed against murine adiponectin (#ACRP303-A, Alpha-

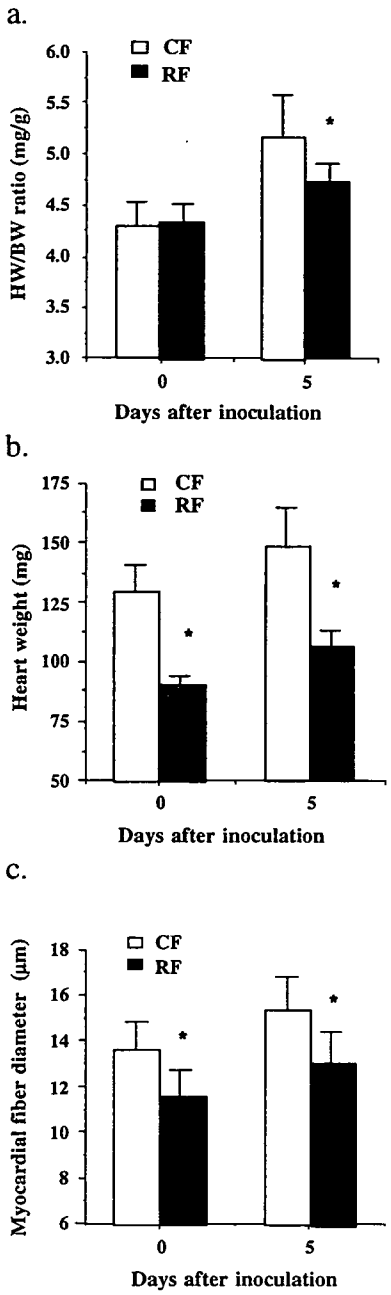

Fig. 2. Heart weight (HW), body weight (BW), and HW/BW ratio in KKAy mice after viral inoculation. HW/BW ratio and HW were significantly lower in RF than in CF ($P<0.01$). (Fig. 2-a, b). Myofiber diameter was significantly reduced in group RF than in group CF (Fig. 2-c, $P<0.01$).

Diagnostic International Inc., San Antonio, USA). Sections were counterstained with hematoxylin.

2.6. Viral titer in heart

The EMC viral titer in the heart was determined in terms of the viral cytopathic effect, and expressed as the tissue culture mean infectious dose (TCID50). On day 4 after inoculation ($n=3$ for each group), hearts were homogenized in 2 ml MEM. After centrifugation, the supernatants were added into 96-well microtiter plates containing human amnion cells in MEM supplemented with 10% fetal calf serum as described previously [10]. The microtiter plates were observed daily for 5 days for the appearance of any cytopathic effect.

2.7. Comparative expression levels of NF-κB, PPAR-γ, TNF-α, and adiponectin mRNA in heart

RNA extraction for each half of frozen cardiac tissue was performed as described by the manufacturer (RNeasy Mini Kit, QIAGEN Inc., Tokyo, Japan). Application of DNAase was performed during RNA extraction to avoid DNA contamination. The total RNA concentration was determined by measuring the optical density at 260 and 280 nm. Aliquots of 20 ml RNA from each tissue were used for production of cDNA. Comparative expression levels of PPAR-γ, NF-κB, TNF-α, and adiponectin mRNA in cardiac tissue from both groups were determined using quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) as described previously [10]. TaqMan MGB Probe (Applied Biosystems Inc., CA, USA) was applied for real-time PCR. We used a commercially available kit for NF-κB, PPAR-γ, TNF-α and adiponectin RT-PCR (Mm00440945 m1, Mm00456849 m1, Mm00443258 m1 and Mm00456425 m1, Applied Biosystems Inc.). Each threshold cycle number up to 50 cycles (Ct value) within the RT-PCR was examined for each mRNA level. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an endogenous internal standard, and was amplified with specific primers for the number of cycles. A negative control without template cDNA was always included. ΔCt values referred to differences between the Ct values for each target gene and the GAPDH gene. After confirming that the efficiency of amplification of each

Table 2
Histopathological and immunohistochemical findings in heart in KKAy mice

	CF in KKAy mice		RF in KKAy mice	
Number	5	5	5	5
Days after inoculation	0	5	0	5
Histopathological scores of heart				
Myocardial injury	0	2.1+0.6		1.1+0.4*
Inflammation	0	0.8+0.2		0.3+0.2*
Immunohistochemical staining of adiponectin				
Myocytes, myofibrils	+1	+2	+2	+3
Myocytes,	+1	+1	+1	+2
Epimysium	–	–	–	–
Interstitial cells	–	+1	–	+1
Inflammatory cells	+2	+2	+2	+2
Endothelial cells	–	–	–	–
Smooth muscle cells	–	–	–	–
Adventitia				

* $P<0.05$ vs. CF on same day.

molecule and GAPDH transcripts was approximately equal, the amount of NF-κB, PPAR-γ, TNF-α, or adiponectin transcript relative to the GAPDH transcript was determined using the comparative Ct method described in Perkin Elmer Applied Biosystems User Bulletin #2. Data are expressed as the fold-increase relative to the baseline value in the heart in mice with CF without viral inoculation.

2.8. Assay of natural killer (NK) cell activity

NK cell activity was assayed by a standard ^{51}Cr-release assay as described previously [10]. Briefly, YAC-1 tumor cells, which are NK-susceptible target cells, were labeled with ^{51}Cr and diluted to a concentration of 1×10^5 cells/ml in RPMI 1640 culture medium containing 10% fetal bovine serum. Spleen cells from mice (4 from each group) were suspended in the same medium and used as effector cells. Both the spleen cells and target YAC-1 cells were placed in round-bottomed 96-well microtiter plates at an effector cell/target cell ratio of 100:1, and incubated at 37 °C in a humidified chamber containing 5% CO_2 for 4 h. The cells were then harvested and counted in a gamma counter, and these procedures were repeated three times. The percentage of specific lysis was calculated as follows:

$$\% \text{ Lysis} = \text{Experimental release} - \text{Maximum spontaneous release} - \text{Spontaneous release} \times 100$$

2.9. Statistics

Data are presented as mean+S.D. Kaplan-Meier test was used to analyze differences in survival. The differences in scores of myocardial changes were examined by two-way analysis of variance. Scheffes' F test and Bonferroni/Dunn analysis were performed for confirmation. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Survival

Survival was significantly improved in group RF as compared with group FF after viral inoculation ($P<0.01$, Fig. 1).

3.2. Organ weights

BW and HW in group RF on day 0, 3 and 5 after viral inoculation were significantly lower ($P<0.01$, Table 1, Fig. 2-b) than those in group CF. HW/BW ratio in group RF on day 0 was the same as that in group CF, but that in group RF on day 5 was significantly lower than that in group CF ($P<0.01$) (Fig. 2-a). Spleen weight/BW and thymus weight/BW ratios in group RF on day 0 were significantly lower ($P<0.01$) than those in group CF, but those on day 5 were not significantly different between the two groups. Liver weight/BW ratio on both days 0 and 5 was lower in group RF compared with group CF (Table 1).

3.3. Pathological findings

Myocardial necrosis with immune cell infiltration was observed in both groups on day 5. However, the score of myocardial necrosis on day 5 in group RF was significantly lower than that in group CF (Table 2, Fig. 3). Myofiber

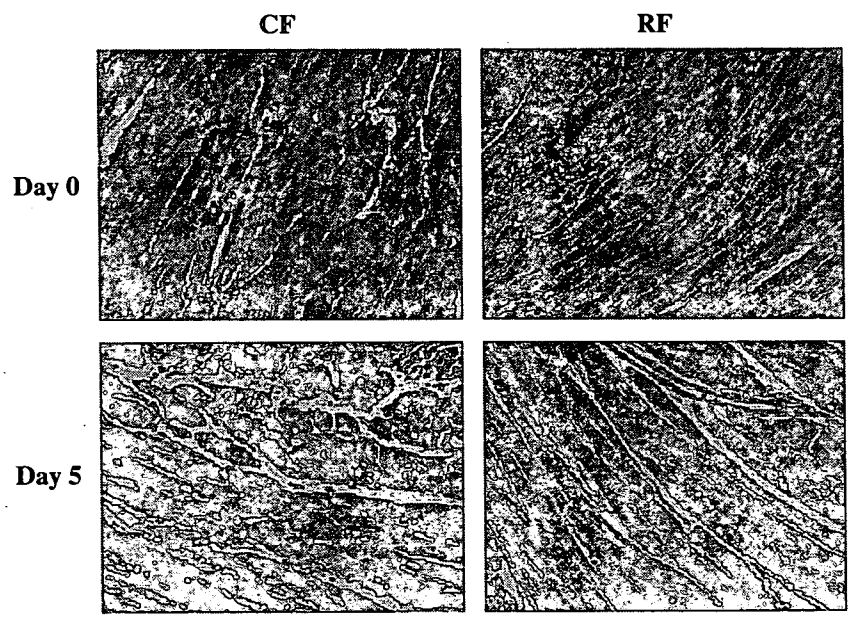

Fig. 3. Pathological findings in heart of KKAy mice after viral inoculation. Myocardial necrosis with immune cell infiltration was observed in both groups on day 5. However, myocardial necrosis on day 5 was reduced in group RF compared with group CF.

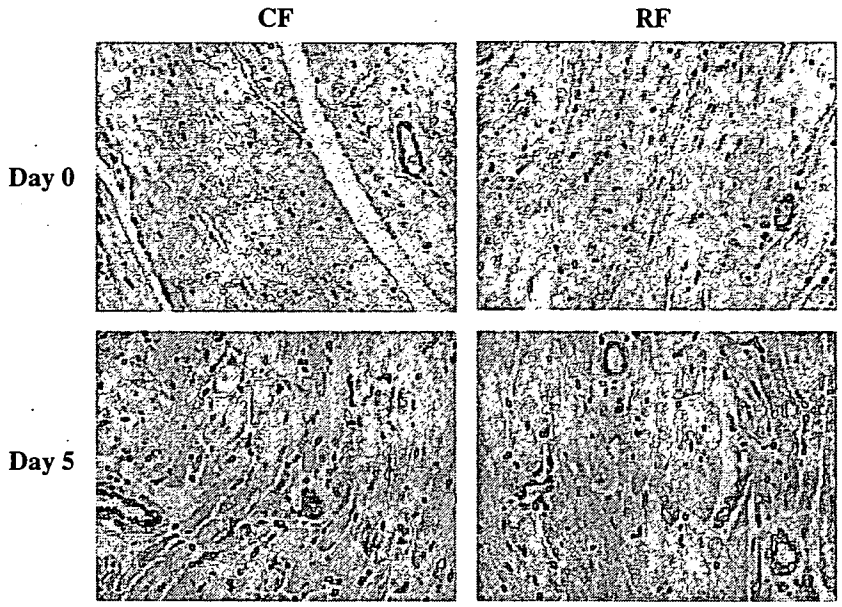

Fig. 4. Immunohistochemical findings. Localized expression of adiponectin in the heart was shown. Endothelial cells and myofibers showed positive expression in both groups on day 0.

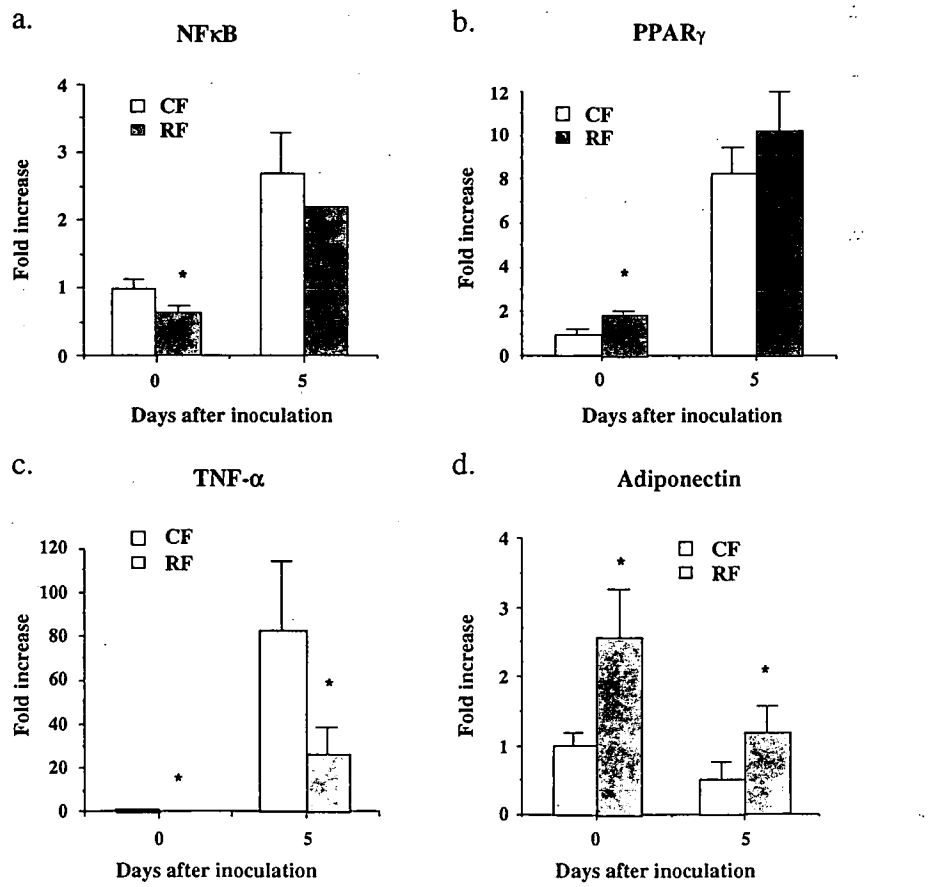

Fig. 5. Expression of cardiac NF-κB, PPAR-γ, TNF-α, and adiponectin mRNA by RT-PCR. The expression level of NF-κB mRNA in the heart in group RF was significantly lower than that in group CF on day 0 ($P<0.05$, $n=3$), those of PPAR-γ and adiponectin mRNA were significantly higher in group RF on day 0 ($P<0.05$, $n=3$), and that of adiponectin mRNA was significantly higher in group RF, but that of PPAR-γ mRNA was the same on day 5. TNF-α mRNA expression in the heart was significantly lower in RF than in CF on days 0 and 5 ($P<0.05$, $n=3$).

diameter was significantly smaller in group RF than in group FF ($P<0.05$, Fig. 2-c).

3.4. Immunohistochemical findings

Localized expression of adiponectin in the heart is shown in Table 2. Endothelial cells and myofibers showed positive adiponectin expression in both groups before viral infection (Fig. 4). Myofibers in group RF were slightly more positive than those in group CF. After infection, myofibers and the epimysium were highly positive and inflammatory cells were positive.

Immunohistochemical staining was scored by assessing the staining intensity. A staining intensity was rated as slight (+1), moderate (+2), and intense (+3) based on the intensity of staining uniformly visualized over the whole section. Back ground was considered as negative (0).

3.5. Expression of cardiac NF-κB, PPAR-γ, TNF-α, and adiponectin mRNA determined by RT-PCR

The expression level of NF-κB mRNA in the heart in group RF was significantly lower than that in group CF on day 0 ($P<0.05$, $n=3$), and those of PPAR-γ and adiponectin mRNA were significantly higher in group RF on day 0 ($P<0.05$, $n=3$). On day 5, the expression level of TNF-α mRNA in the heart in group RF was still significantly lower than that in group CF ($P<0.05$, $n=3$, Fig. 5), and that of adiponectin mRNA was significantly higher in group RF, but PPAR-γ mRNA was the same in both groups (Fig. 5).

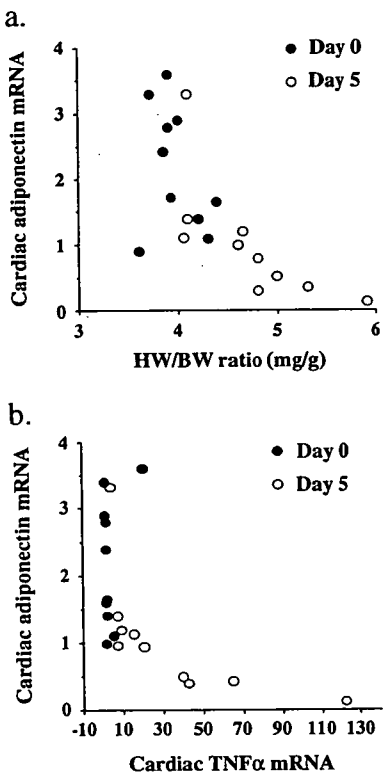

Fig. 6. Correlation of cardiac adiponectin and heart weight (HW)/body weight (BW) ratio, or TNF-α mRNA. There was a significant negative correlation between cardiac adiponectin mRNA and HW/BW ratio ($r=-0.493$; $P=0.0124$), and between cardiac adiponectin mRNA and TNF-α mRNA ($r=-0.555$; $P=0.0097$).

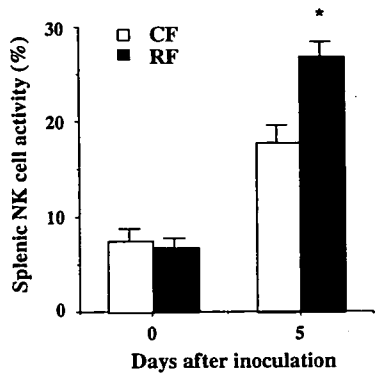

Fig. 7. Natural killer cell activity in spleen. NK cell activity in the spleen was significantly higher in group RF than in group FF on day 4 ($P<0.05$, $n=4$).

A significant negative correlation was found between cardiac adiponectin mRNA level and HW/BW ratio on day 0, 3 and day 5 after viral inoculation ($r=-0.701$; $P=0.0026$). There was also a significant negative correlation between cardiac adiponectin mRNA and TNF-α mRNA levels ($r=-0.555$; $P=0.0097$, Fig. 6).

3.6. Viral titer in heart

On day 4, the viral titer in the heart in group RF was significantly lower than that in group CF (3.2+0.2 vs. 3.6+0.2 TCID50/mg, $P<0.05$, $n=3$ for each).

3.7. NK cell activity in spleen

NK cell activity in the spleen in group RF was significantly higher than that in group CF on day 4 (27± 4% vs. 18±3%, $P<0.05$, $n=4$ for each, Fig. 7). There was no significant difference in NK cell activity on day 0.

4. Discussion

The present study showed that a restricted diet in obese KKAy mice improved the survival rate, decreased cardiac viral titer, increased splenic NK cell activity and reduced both myocardial necrosis and lymphocytic infiltration in mice with viral myocarditis. In addition, a reduced diet suppressed cardiac hypertrophy at the morphological and cellular levels. Therapeutic modulation of cardiac adiponectin might provide benefit through a cardioprotective effect against acute heart failure due to viral myocarditis in obese subjects. Cardiac adiponectin could be induced by activated PPARγ mRNA and reduced expression of NF-κB in the myocardium of mice with food restriction.

Obesity is increasingly viewed as an inflammatory state. General enhancement of adipose tissue-derived cytokine expression may be another plausible mechanism for the

inflammation/metabolic syndrome relationship. The role of adipose tissue as an endocrine organ capable of secreting a number of adipose tissue-specific or enriched hormones, known as adipokines, is gaining appreciation. An imbalance between increased inflammatory stimuli and decreased anti-inflammatory mechanisms is an intriguing working hypothesis. Adiponectin is negatively correlated with obesity, especially visceral fat masses and with lipid metabolism [11]. Adiponectin also suppresses activation of NF-κB [12]. Indeed, overweight rats showed higher PPARγ combined with inhibition of NF-κB [13].

Obesity frequently leads to left ventricular hypertrophy [14]. It associated with a chronically high cardiac workload due to the need to supply more blood to peripheral tissues. The high cardiac output is mainly a consequence of the greater requirements of increased lean body mass, and is maintained by an increased stroke volume and high normal heart rate, which is sustained by an increase in ventricular mass. The increase in left ventricular mass also implies an increase in non-muscular tissue, which plays a role in the development of electrical abnormalities, heart failure and sudden death. Obesity per se is a major risk factor for heart failure [14]. Our results showed that a reduced diet induced a reduction in cardiac hypertrophy in obese KKAy mice without infection. Therefore, weight reduction leads to decreased left ventricular mass and reduces the risk of cardiac death. A reduction of cardiac hypertrophy by adiponectin has been reported. Shibata et al. showed that adiponectin inhibits hypertrophic signaling in the myocardium through activation of AMP-activated protein kinase [15]. They also suggested that adiponectin could be used to treat cardiac hypertrophy such as hypertrophic cardiomyopathy associated with obesity-related diseases. Therefore, reduced diet in obese mice could inhibit cardiac hypertrophy through the induction of cardiac expression of adiponectin mRNA.

Food restriction and subsequent weight loss increased adiponectin expression of visceral fat in an obese rat model [16]. Previous observations showed that plasma adiponectin is decreased in obese rodents [17], and chronic caloric restriction is able to enhance the circulating level of this protein [18]. Also, in humans, weight loss increases plasma adiponectin level despite a reduction of adipose tissue [9]. All these observations taken together suggest that adiponectin expression is under feedback inhibition in obesity. The effect of weight loss on the metabolic syndrome attributes cardiovascular function [19]. In a cohort of outpatients, a higher body mass index was associated with higher mortality risk. Overweight and obese patients had a higher risk of death compared with those with a healthy weight [20]. Weight loss, particularly in persons who are severely obese, can improve cardiac structure and function [3]. In our results, adiponectin was expressed in the heart and was upregulated by food restriction in an obese mouse model.

Cardiac expression of adiponectin has been reported in autopsy cases [21,22]. Adiponectin was distributed in the interstitium of infarcted lesions at an early stage, and was present linearly both along the border of vital myocardium and at the periphery of surviving cardiomyocytes around the lesion in the granulation stage [21]. We have reported that adiponectin and its receptor were expressed in injured myocardium in autopsy cases of cardiomyopathy [22]. A recent report showed that adiponectin inhibited NF-κB activation and increased PPARγ expression in adipocytes [12]. Therefore, increased expression of cardiac adiponectin in food-restricted mice may suppress local inflammation via its regulation of the NF-κB and PPARγ transcription factors.

PPARγ is a transcription factor belonging to the nuclear receptor superfamily and is present in a variety of cells including myocardium [23,24]. Myocardium uses either fatty acid or glucose oxidation as the main energy source. Fatty acid oxidation is transcriptionally regulated by the fatty acid activated PPAR superfamily. We suggest that PPARγ may directly regulate cardiac inflammation, which plays a critical role in the progression of cardiac remodeling and dysfunction. A previous report showed that PPAR activators inhibit lipopolysaccharide-induced TNF-α expression in neonatal rat cardiac myocytes, and that this effect of PPARγ would be beneficial in preventing the development of congestive heart failure [23]. In the present study, we demonstrated that enhanced expression of PPARγ suppresses the development of heart failure due to viral myocarditis, associated with induced expression of cardiac adiponectin and suppressed cardiac TNF-α expression. Indeed, it has been reported that PPAR ligands could downregulate inflammatory responses, and stimulation of PPARγ blocked viral replication and TNF-α production in blood cells [25,26].

NF-κB is a central mediator of host immune responses, and activation of NF-κB enhances viral replication and evasion of immune responses [27]. Our data showed lower NF-κB mRNA in the heart of food-restricted mice than in obese mice. Decreased expression of cardiac NF-κB could reduce viral replication and inflammation in the heart through weight loss by food restriction. In a recent report, NF-κB was required for the hypertrophic response of cardiomyocytes in vitro [28]. Indeed, our data showed decreased heart weight and lower myofiber diameter by food-restriction in comparison with obese mice with downregulation of NF-κB expression.

Impaired immune function linked to obesity has been shown in both human and animal studies [29]. Diet control is also effective for impaired immune function including NK cell activity in obese animals [30]. A study of obese humans showed that removal of a significant amount of subcutaneous fat tissue had no effect on inflammatory markers [31]. Genesis of inflammatory reaction may be not due to adipose tissue but due to other organs, such as the heart, in obese subjects.

Cardiac expression of adiponectin mRNA was negatively correlated with cardiac TNF-α mRNA expression in this study. TNF-α is secreted primarily by myocytes and macrophages after injury [32]. Elevation of TNF-α has been shown to contribute to ventricular dysfunction, using TNF-α knockout mice [33]. A previous study has demonstrated that cardiac-specific expression of TNF-α results in

myocardial inflammation, cardiac hypertrophy, progressive dilatation, and increased apoptosis, leading to heart failure and death [34]. TNF-α play an important role in modulating left ventricular dysfunction [35]. A recent study showed a relation between adiponectin and TNF-α in adiponectin knockout mice [36], which demonstrated higher TNF-α expression in adipose tissue, and the administration of adiponectin resulted in a decrease in TNF-α expression. Thus, TNF-α and adiponectin are antagonists of each other, or one cytokine control the expression of the other [37]. Weight loss altered the cytokine expression in heart tissue, where adiponectin was inversely expressed with TNF-α.

The therapeutic implication of diet control in obese subjects is the prevention of acute heart failure with viral myocarditis with cardiac expression of adiponectin and TNF-α. Diet control is also beneficial in regulation of the immune reaction against cardiotropic viral infection.

Acknowledgements

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