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Oral Administration of Candesartan Improves the Survival of Mice with Viral Myocarditis through Modification of Cardiac Adiponectin Expression

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Abstract
Purpose We examined the effects of the angiotensin II receptor type1 blocker candesartan on myocarditis injury in a murine model of acute myocarditis. We hypothesized that candesartan improves cardiac damage by inducing cardiac expression of adiponectin.
Methods and results We examined changes in heart failure caused by myocarditis in mice by candesartan based on induction of cardiac adiponectin expression. We intraperitoneally injected encephalomyocarditis virus in C3H mice, then orally administered candesartan (10 mg/kg/day) or vehicle (control). The 7 day survival rate was 18% in the control group, but 60% in the candesartan group. The heart weight/body weight ratio in the candesartan group was significantly lower than in the control group. Circulating adiponectin concentrations on day 7 were significantly higher in the candesartan group compared with the control group (7.91±0.61 vs. 6.04±2.26 μg/ml, $P<0.05$). Comparative expression of cardiac adiponectin mRNA in the candesartan group was significantly higher than in the control group on day 7 (55.4±41.3 vs. 5.3±7.7, $P<0.05$). Immunohistochemical staining and in situ hybridization showed that cardiac expression of adiponectin protein and mRNA was present in the candesartan group on day 7.
Conclusion Oral administration of candesartan improves survival and decreases myocardial damage in mice with viral myocarditits and induces expression of cardiac adiponectin. The induction of adiponectin might provide cardioprotective effects against acute heart failure due to viral myocarditis.

Key words candesartan · myocarditis · adiponectin · acute heart failure

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Introduction

Acute myocarditis is a potentially lethal disease, and frequently precedes the development of dilated cardiomyopathy [1]. A murine model of vial myocarditis induced by the encephromyocardits (EMC) virus is associated with a high incidence of severe myocarditis, congestive heart failure, and dilated cardiomyopathy [2].

Recently, we and others have reported that treatment with angiotensin II type1 blockers (ARB) reduces viral-mediated myocardial injury [3, 4]. ARB has been shown to have anti-inflammatory properties in vitro and animal studies. In the setting of cardiovascular diseases, ARB reduce the expres-

sion of inflammatory cytokines including tumor necrosis factor-α (TNF-α) and its transcriptional factors [5–7].

Adiponectin is an anti-inflammatory and anti-atherogenic cytokine [8]. ARB treatment increases circulating adiponectin concentrations in human [9] and in mice [10, 11]. ARB treatment increases circulating adiponectin mRNA in fat tissue in hypertensive rats [12]. Our previous study showed that acute myocarditis is unknown. The purpose of this study was to examine the role of the ARB, candesartan, in mice with acute viral myocarditis, focusing on its effect on cardiac expression of adiponectin as well as cardiac TNF-α mRNA.

Materials and methods

Animal model

Eight-week-old C3H mice were purchased from Clea (Tokyo, Japan). At 9 weeks of age the mice were divided randomly into two groups (n=30 in each group) for either candesartan treatment at a daily dose of 10 mg/kg for 7 days starting from on the day of viral infection (candesartan group) or treatment with vehicle alone (control group). Uninfected mice (n=5) also were studied. The D variant of the encephalomycarditis (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 injected intraperitoneally with 500 plaque-forming units of EMC virus in 0.1 ml of saline. No virus was detected by viral titer assay on day 14. The animals were placed in isolation cages and fed a standard diet and water. The University Committee on Animal Care of Kanazawa Medical University approved the entire experimental protocol.

Measurement of plasma adiponectin

Blood sample were obtained from all mice before killing by cervical distillation. All serum samples were stored at −80°C until analysis. The circulating concentrations of adiponectin were measured by enzyme linked immunosorbent assay (ELISA), according to the manufacturers' instructions (ELISA kit for mouse or rat adiponectin, Otsuka Pharmaceutical, Tokyo, Japan). This kit used for adiponectin demonstrated a sensitivity, intra-assay variation, and cross-reactivity of 0.25 ng/ml, <10%, and no responses for specimens from other animals including sheep, respectively.

Histologic examination

The body weight and heart weight were determined. One half of each organ was fixed in 10% buffered formalin for tissue staining and immunohistochemical studies; the other half was frozen for molecular analysis. Transverse sections of ventricular myocardium were graded for the severity of necrosis and mononuclear cell infiltration as follows: grade 1, lesions involving <25% of ventricular myocardium; grade 2, lesions involving 25–50% of the myocardium; grade 3, lesions involving 50–75% of the myocardium; and grade 4, lesions involving 75–100% of the myocardium.

Imunohistochemical examination

To visualize the present and anatomic localization of adiponectin within the myocardium, immunohistochemical studies were performed. Imunohistochemistry was performed by the avidin biotin complex method (Vectastain ABC kit, Vector laboratories, Burlingame, CA) as previously described [13]. To minimize background staining, all sections first were 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-Diagnostic International, San Antonio, Tx) or against murine TNF-α (#AF-410-NA, R&D systems, Minneapolis, MN). Sections were counterstained with hematoxylin.

In situ hybridization for adiponectin mRNA in the heart

To investigate the localization of adiponectin mRNA in the heart in heart, in situ hybridization was performed on myocardial sections using an adiponectin antisense RNA probe as reported previously [14].

Comparative expression levels of adiponectin and TNF-α mRNA in hearts

RNA extraction from half the frozen cardiac tissue was performed as described by the manufacturer (RNeasy Mini Kit, QIAGEN, Tokyo, Japan). DNAase treatment was

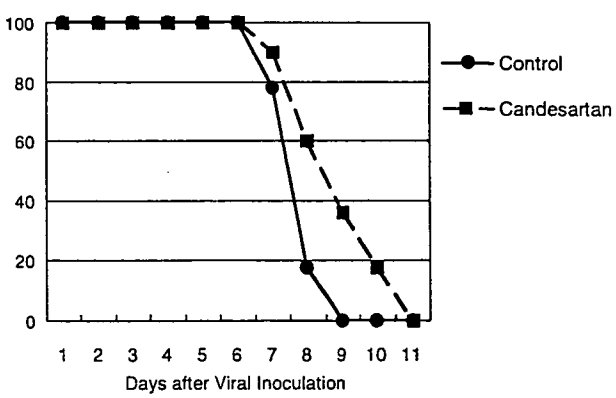

Fig. 1 Survival in mice after viral infection. Survival was significantly (P<0.05) improved in the Candesartan group

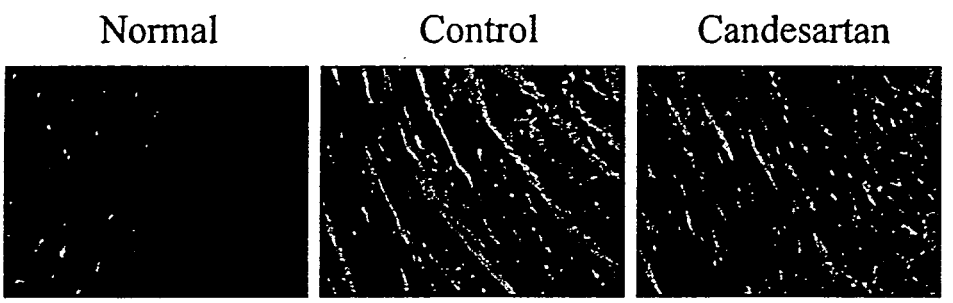

Fig. 2 Hematoxylin and eosin stains shows that myocardial injury with immune cell infiltration was observed in the Control group on day 7. However, the degree of myocardial injury was reduced on day 7 in the Candesartan group. Myofiber diameter was reduced in the Candesartan group compared with the Control group

performed during the RNA extraction to avoid DNA contamination. The total RNA concentration was determined by measuring the optical density of the sample at 260 and 280 nm. Aliquots of 20 μl RNA and TNF-α mRNA in cardiac tissue from both groups were determined using a quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) as described previously [13]. We used TaqMan MGB probe (Applied Biosystems, San Diego, CA) for the RT-PCR. We used a commercially available kit for adiponectin and TNF-α RT-PCR (Mm00443258 ml and Mn00456425 ml, Applied Biosystems, San Diego, CA). The glyceraldehydes-3-phosphate dehydrogenase (GAPDH) gene was used as an endogenous internal standard, and was amplified with specific primers. A negative control without templates cDNA was always included. The ΔCt values referred to differences between the Ct value for the target gene and the GAPDH transcripts were approximately equal, the amount of the adiponectin or TNF-α transcript relative to the GAPDH transcript was determined using the comparative Ct method as described in Perkin Elmer Applied Biosystem User Bulletin #2. Values are expressed as fold-increases relative to the baseline values for hearts frome uninfected normal mice.

Statistics

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

Results

Survival

Survival was significantly improved in the candesartan group compared to the control group after viral infection ($P<0.05$, Fig. 1).

Organ weights

The body weight was significantly higher in the control group than in the uninfected normal mice and the heart weight was significantly greater in the control group than in

Table 1 Heart weight, histologic findings, and cytokine mRNA expression in mice 7 day after viral infection

	Normal	Control	Candesartan
Number	5	7	7
Body weight (g)	18.3±7.7	15.5±4.2[a]	17.5±3.3[a, b]
Heart weight (mg)	93.5±9.4	112.2±12.9[a]	97.4±10.6[a, b]
Heart weight/body weight ratio (mg/g)	5.1±0.7	7.2±1.1[a]	5.6±0.8[a, b]
Plasma adiponectin (μg/ml)	7.09±1.32	6.04±2.26[a]	7.91±0.61[a, b]
Histipathologic scores of the heart			
Myocardial injury	ND.	2.4±1.0[a]	1.5±0.7[a, b]
Inflammation	ND.	2.1±0.9[a]	1.1±0.4[a, b]
Myofiber diameter(μm)	12.6±1.2	15.4±1.9[a]	13.8±1.4[a, b]
Adiponectin mRNA	1±0.2	5.3±7.7[a]	55.4±41.3[a, b]
TNF-α mRNA	1±0.3	63.6±8.0	5.2±2.3[a, b]

Values are mean ± SD. [a] $P<0.05$ vs. normal group, [b] $P<0.05$ vs. Control
ND. non detectable

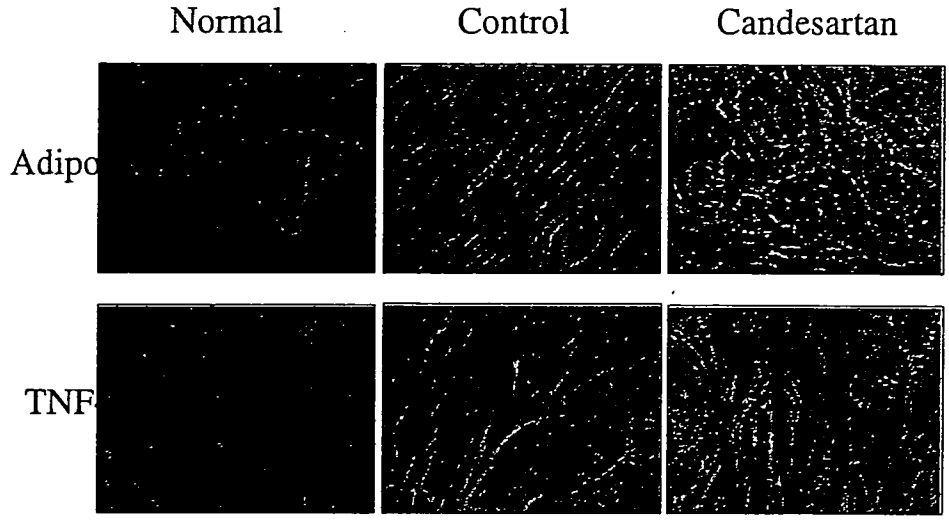

Fig. 3 Immunohistochemical staining in the heart of uninfected normal mice (Normal), untreated control mice on day 7 after viral infection (Control), and candesartan-treated mice (Candesartan). Immunohistochemical staining showed that cardiac expression of adiponectin and TNF-α was almost absent in normal mice, except in the vascular wall for adiponectin was strongly positive in the myocardium in the Candesartan group. Injured myocytes expressed TNF-α in the Control group on day 7, and endothelial cells and myofibers were weakly positive in the Candesartan group

normal mice (Fig. 2). The body weight and heart weight in the candesartan group on day 7 after viral infection was significantly lower than in the control group ($P<0.05$, Table 1).

Histological findings

Myocardial necrosis with immune cell infiltration was observed in both groups on day 7. However, the myocardial necrosis score on day 7 in the candesartan group was significantly lower than in the control group (Table 1). Myofiber diameter was significantly reduced in the candesartan group compared to the control group ($P<0.05$, Table 1).

Immunohistochemical findings

Cardiac expression of adiponectin protein was present in the candesartan group on day 7 but the finding was only slightly positive in the control group (Fig. 3). Staining in the normal heart was negative. Staining for cardiac TNF-α protein was strongly positive in the control group but was weakly positive in the candesartan group on day 7. In normal mice, staining for cardiac TNF-α protein was negative.

In situ hybridization for adiponectin mRNA in the heart

The genomic expression of adiponectin mRNA in transverse ventricular sections largely overlapped with that of adiponectin protein observed using the immunohistochemical and RT-PCR methods. Infected myocytes in the candesartan group were moderately to strongly positive on day 7. However, the absence of adiponectin mRNA was noted in myocytes from normal uninfected mice. There was hardly any adiponectin signal in the myocardium from control mice at the same time points (Fig. 4).

Expression of cardiac adiponectin and TNF-α mRNA by RT-PCR

Cardiac adiponectin mRNA expression was significantly higher in the candesartan group than in the control group. Cardiac TNF-α mRNA expression was significantly lower

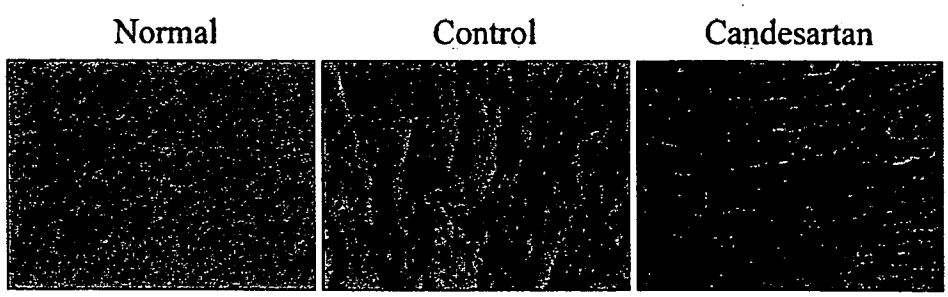

Fig. 4 Detection of adiponectin mRNA (normal) by in situ hybridization in myocardium from mice on day 7 after viral infection. Adiponectin mRNA was absent in myocytes from uninfected normal mouse. A moderate to strong signal for adiponectin mRNA was detected in myocytes from Candesartan treated mice 7 day after viral inoculation. There was a slightly positive adiponectin signal in the myocardium from infected control mice at the same time

in the candesartan group than in the control group (Table 1). Cardiac adiponectin and TNF-α mRNA expression were significantly increased in the control group compared to normal mice.

Discussion

We have shown that oral administration of candesartan improves survival rates, decreases myocardial necrosis, and decreases lymphocyte infiltration in mice with viral myocarditis. In addition, candesartan treatment increases the cardiac expression of adiponectin, which might provide cardioprotective effects against acute heart failure due to viral myocarditis by reducing the expression of cardiac TNF-α.

Adiponectin protein was distributed in the interstitium of infarcted lesions at an early stage. Adiponectin was found both along the border of viable myocardium and at the periphery of surviving cardiomyocytes around lesions at the granulative stage [15]. There was minimal expression of cardiac adiponectin by ISH after viral infection, the infected myocardium showed moderate to strong signal for adiponectin mRNA in myocytes from mice treated with candesartan on day7 in comparison with untreated myocardium. We also reported that adiponectin and its receptor are expressed in the injured myocardium in patients with cardiomyopathy [13]. Although the role of adiponectin in the myocardium is not clear, enhanced expression of cardiac adiponectin on the protein and mRNA level by candesartan treatment would be beneficial in the suppression in murine model of viral myocarditis.

Candesartan therapy has already been reported to reduce cardiac necrosis and inflammation in a murine model of viral myocarditis by Tanaka et al. [16]. However, they showed that the survival of mice receiving 10 mg/kg of candesartan did not improve significantly. Our data showed the improved survival by the same dose of candesartan. This difference may depend on haplotype of mice, which may affect the susceptibility of EMC virus infection.

Candesartan treatment reduces inflammation and lowers plasma CRP concentration in hypertensive patients [17]. Candesartan increases circulating adiponectin concentration in human [9, 18] and in diabetic mice [11]. Candesartan also increases adiponectin mRNA in fat tissue in hypertensive rat [12]. Our data showed that enhanced expression of myocardial adiponectin mRNA and protein was identified with the reduction of myocardial injury through candesartan treatment in a murine model of viral myocarditis. Candesartan could induced cardiac adiponectin expression in acute heart failure.

TNF-α is secreted primarily by myocytes and macrophages after injury [19]. Increased TNF-α expression contributes to extending ventricular dysfunction [20]. Previous studies have demonstrated that cardiac-specific expression TNF-α results in myocardial inflammation, cardiac hypertrophy, progressive dilation, and increased apoptosis, which leads to heart failure and death [21]. TNF-α may play an important role in modulating left ventricular dysfunction [22]. A recent study described the relationship between adiponectin and TNF-α in adiponectin knockout mice [23]. Adiponectin knockout mice demonstrated higher TNF-α expression and the administration of adiponectin resulted in decrease in TNF-α expression. Thus, TNF-α and adiponectin may be antagonist of each other or one cytokine may control the expression of the other [24].

We conclude that oral administration of candesartan is beneficial for the prevention of viral myocarditis through cardiac expression of the anti-inflammatory cytokine, adiponectin, which suppresses expression of the inflammatory cytokine, TNF-α. Further studies will be needed to clarify the cardiac adiponectin signaling by using agents to modify adiponectin regulation.

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Candesartan improves myocardial damage in obese mice with viral myocarditis and induces cardiac adiponectin

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Abstract

Purpose: To clarify the mechanism of the effects of angiotensin II receptor type 1 antagonist, candesartan, upon cardiac adiponectin in the combination of myocarditis with obesity, we examined obese KKAy mice with acute viral myocarditis treated by candesartan and investigated cardiac adiponectin regulation.
Methods: Mice were divided into candesartan early treatment group (Can-early) receiving orally candesartan at daily dose of 10 mg/kg 7 days starting before viral inoculation and then 7 days; candesartan late treatment group (Can-late) or vehicle (Vehicle) receiving candesartan starting simultaneously with viral inoculation and then 7 days. Encephalomyocarditis virus was used to induce the acute viral myocarditis. Differences in myocardial damages, serum adiponectin and myocardial expression of adiponectin, tumor necrosis factor-α (TNF-α), CCAAT/enhancer binding proteinα (C/EBPα) and peroxisome proliferator-activated receptor γ (PPAR-γ) and nuclear factor-κB (NF-κB) mRNA among three groups were determined on days 0, 4 and 7 after viral inoculation.
Results: Mice in Can-early and Can-late groups showed reduced myocardial necrosis and cellular infiltration as compared with those in the Vehicle. On day 4 the circulating adiponectin levels were significantly higher in Can-early than those in Vehicle. Mice in Vehicle had significantly reduced in myocardial adiponectin mRNA after viral myocarditis. Cardiac adiponectin mRNA was significantly higher in Can-early and in Can-late than in Vehicle on days 4 and 7. Cardiac C/EBPα in Can-early and Can-early groups was significantly increased on day 4. Myocardial NF-κB and TNF-α mRNA in Can-early and Can-late groups were significantly reduced on day 7.
Conclusion: Candesartan treatment improved myocardial injury in obese mice with acute viral myocarditis and induced expression of cardiac adiponectin with the induction of C/EBPα as well as the reduction of cardiac NF-κB and TNF-α.

Keywords: Angiotensin II receptor antagonist; Acute viral myocarditis; Adiponectin; Obesity

1. Introduction

Acute myocarditis is a potentially lethal disease, and frequently precedes the development of dilated cardiomyopathy, whose pathogenesis is not completely understood and whose prevalence is likely underestimated [1,2]. Encephalomyocarditis (EMC) virus, a picornavirus, can cause severe

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myocarditis in experimental animals. The disease is characterized by myocardial necrosis and inflammation in the acute stage followed by myocardial fibrosis and hypertrophy in the chronic stage [3,4]. The murine models of viral myocarditis induced by the EMC virus are a high incidence of severe myocarditis, congestive heart failure and dilated cardiomyopathy [5].

Angiotensin II is a true cytokine that regulates cell growth, inflammation, and fibrosis [6], and plays an important role in the pathophysiology of various cardiovascular disorders, such as hypertension, atherogenesis [7] and viral myocarditis [5,8,10]. A growing body of evidence shows that angiotensin II type 1 (AT1)-receptor blockers inhibit cardiac hypertrophy and remodeling, and prevent progression of systolic heart failure, thereby reducing cardiac morbidity and mortality [9,10], protected the heart against ischemia-reperfusion injury and reduced myocardial damage during myocarditis [5,8]. Candesartan, a nonpeptide AT1 receptor antagonist, is generated from the prodrug candesartan cilexetil during gastrointestinal absorption [11].

Adiponectin is an approximately 30-kDa protein that circulates in plasma as multimeric complexes at relatively high concentration and plays a crucial role in the association among obesity, type II diabetes [12]. Our previous study showed that adiponectin was expressed in human myocardium [13]. Recent intensive research on obesity has shown that adiponectin mediates many of its cardiovascular and metabolic complications, although the pathogenic relationships among obesity, metabolic syndrome, and cardiovascular complications remain poorly understood [14].

It has been reported that candesartan increases circulating adiponectin concentrations in human [15] and in diabetic mice [16]. Candesartan also increases adiponectin mRNA of fat tissue in hypertensive rats [17]. However, the role of candesartan in obese mice with acute heart failure is unknown. We assessed the role of an angiotensin II receptor antagonist, candesartan, in obese KKAy mice with acute viral myocarditis, focusing on its effect on expression of cardiac adiponectin mRNA as well as cardiac tumor necrosis factor-α (TNF-α) mRNA. In addition, we examined the induction of two major adiponectin transcription factors CCAAT/enhancer binding proteinα (C/EBPα) and peroxisome proliferator-activated receptor γ (PPAR-γ) and TNF-α transcription factor nuclear factor-κB (NF-κB) in the myocardium. We suggest that candesartan may enhance cardiac adiponectin with reduction of TNF-α through the modification of their transcription factors.

2. Materials and methods

2.1. Animals and treatments

Nine-week-old female obese and type II diabetes KKAy mice weighing 35–40 g were purchased from Clea Japan Inc. (Tokyo, Japan), and maintained with food and water available freely at the animal center of Kanazawa Medical University. All mice were randomly divided into three groups: candesartan early treatment group (Can-early, $n=12$) receiving candesartan at daily dose of 10 mg/kg. 7 days starting before the viral inoculation and then 7 days; candesartan late treatment group (Can-late, $n=12$), and vehicle (Vehicle, $n=12$) receiving candesartan or vehicle starting simultaneously with viral inoculation and then 7 days. Candesartan (TCV-116, lot No. HB982) was provided by Takeda Chemical Industries, Ltd. (Osaka, Japan). This agent was dissolved with Arabic gum in distilled water, and diluted with water to appropriate concentration. Arabic gum alone dissolved with water was used as a vehicle. Candesartan was orally administrated at dose of 10 mg/kg with a feeding needle down the throat once daily. Ethical approval for this study was obtained from the Animal Experimental Committee in Kanazawa Medical University.

2.2. Virus preparation and induction of acute viral myocarditis

A variant of EMC virus was obtained from Y. Seto, Ph. D (Keio University, Tokyo, Japan). Virus preparations were stored at −80 °C in Eagle's minimum essential medium supplemented with 0.1% fetal bovine serum until use. Each mouse was injected intraperitoneally with 0.1 mL of minimal essential medium of stock virus containing an infective dose of 500 plaque forming units for induction of acute viral myocarditis [18]. After the EMC virus infections, the survival rate of each group was monitored during the observation period. Body weight (BW), heart weight (HW) and histopathological score of hearts were examined on days 0, 4 and 7.

2.3. Histopathology examination

At sacrifice, the mice were weighed and then killed by cervical dislocation. Hearts were immediately extracted carefully and weighed, and the ratios of HW per BW were also calculated, half of hearts were fixed for 24 h in 10% buffered formalin for tissue staining and embedded in paraffin. Transverse sections at maximal circumference of the ventricle, stained with hematoxylin and eosin, and examined by the light microscope. The other half hearts were frozen in liquid nitrogen for molecular and stored at −80 °C until use. Myocardium sections were graded for the severity of necrosis and mononuclear cell infiltration blindly by a pathologist from 1 to 4 as described previously: grade 1, lesions involving <25% of myocardium; grade 2, lesions involving 25% to 50%; grade 3, lesions involving 50% to 75%; and grade 4, lesions involving 75% to 100%. The experienced pathologist had no knowledge of our study design. Five high power fields in each myocardium section were selected randomly and blindly counted the infiltrating cells (×400 magnification) as previous described [18].

2.4. Blood sample assay

Before sacrifice, blood samples were obtained from all mice. Blood glucose concentrations were determined by a

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glucose oxidation method utilizing a Fuji Dry Chem System (Medical System Co., Tokyo, Japan). All serum samples were stored at −80 °C until analysis. The circulating levels of adiponectin were measured by enzyme linked immunosorbent assay (ELISA), according to the manufactures' instructions (ELISA kit for mouse or rat adiponectin, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). This kit used for adiponectin levels demonstrated that the limit of sensitivity, the intra-assay variation, and the cross-reactivity were 0.25 ng/mL, less than 10%, and no responses for specimens from other animals including sheep, respectively.

2.5. Immunohistochemical examination

To visualize the presence and anatomic localization of adiponectin within the myocardium, immunohistochemical studies were performed. Immunohistochemistry was performed by the avidin biotin complex methods (Vectastain ABC kit, Vector laboratories, Burlingame, CA) previously described [18]. To minimize the background staining, all sections were first blocked with normal goat serum for 20 min at room temperature. In next, the slides were incubated with an antibody directed against murine adiponectin (#ACRP303-A, Alpha-Diagnostic International Inc. San Antonio, USA) or against murine TNF-α (#AF-410-NA, R&D systems, Minneapolis, MN, USA). Sections were counter stained with hematoxylin.

2.6. Comparative expression of NF-κB, C/EBPα, 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, C/EBPα, 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 [19]. 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 (Applied Biosystems Inc.). Primer sequence and PCR conditions in C/EBPα were as follows: forward, 5′-CAAAGCCAAGAAGTCGGTGGACAA-3′; reverse, 5′-TCATTGTGACTGGTCAACTCCAGC-3′; annealing temperature, 55 °C; cycles, 35; size, 150 bp. 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 same number of cycles. A negative control without template cDNA was always included. D Ct 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 molecule and GAPDH transcripts was approximately equal, the amount of NF-κB, C/EBPα, 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.

2.7. Statistical analysis

All data were expressed as mean ± SD. Statistical analysis of data was done by one-way analysis of variance (ANOVA) for differences in body weight, heart weight; numbers of infiltrating and apoptotic cells in myocardium; serum adiponectin level and myocardial expression of mRNA, and by

Table 1
Effects of candesartan on body weight (BW), heart weight (HW) in KKAy mice with acute viral myocarditis

Groups	N	BW (g)	HW (mg)	HW/BW (%)	Glucose (mg/dL)	Adiponectin (μg/mL)
Day 0						
Vehicle	4	41.2±2.8	137.1±16.0	3.32±0.22	268±52.2	31±4
Can-early	4	38.9±0.9	123.5±6.0'	3.18±0.10	236±51.5	27±4
Can-late	4	41.5±2.8	134.4±16.0	3.36±0.27	251±54.7	29±2
Day 4						
Vehicle	4	36.8±1.9'	152.3±9.74'	4.14±0.13'	333.3±161.5	24±3'
Can-late	4	41.6±1.8	137.0±0.82*	3.30±0.14**	417.8±217.8	33±4*
Can-early	4	37.7±1.1'	125.5±11.6*'	3.32±0.23**	258.8±178.0	27±7
Day 7						
Vehicle	4	31.7±2.8'	144.1±10.8'	4.54±1.80'	262.2±161.5	20±2'
Can-late	4	37.4±3.3'	134.3±2.8	3.61±0.40	278.4±134.4	31±3*
Can-early	4	27.2±7.9'	116.5±19.0'	4.31±0.19'	181.5±161.5	30±2*

KKAy mice were treated with candesartan 10 mg/kg/day or vehicle (Vehicle). On day 4 after viral inoculation the HW and HW/BW ratio in Can-late and Can-early mice were significantly decreased compared with those in the vehicle. *$P<0.01$, **$P<0.001$ vs. Vehicle on the same day, '$P<0.01$ vs. Vehicle on day 0.

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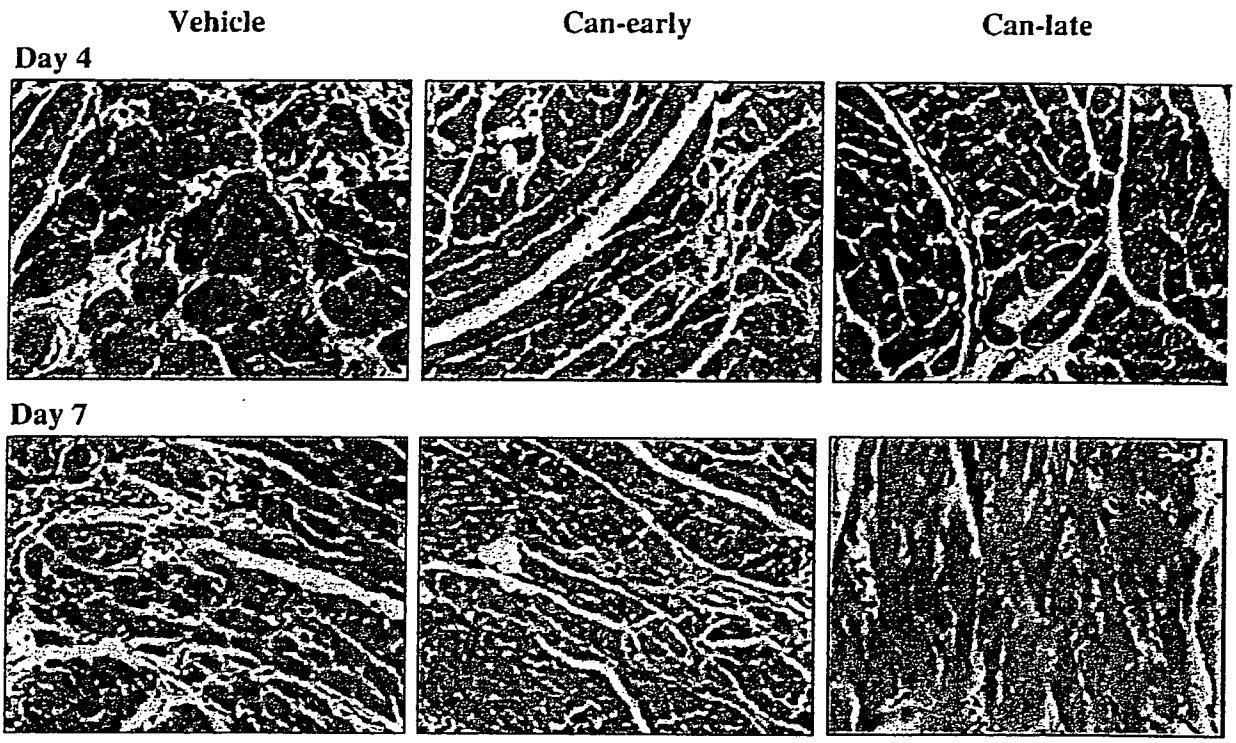

Fig. 1. Pathological findings of the heart of KKAy mice after virus inoculation. Myocardial necrosis with immune cell infiltration was observed in both groups on days 4 and 7. However, myocardial necrosis was reduced in Can-early and Can-late groups compared with the group Vehicle.

Kruskal–Wallis test for difference in cardiac histological score, Kaplan–Meier analysis to analyze the survival rate of mice. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Body weight and heart weight

Administration of candesartan had no obvious effect on the BW on day 0, day 4 and day 7 among the Can-early, Can-late and Vehicle groups (Table 1). However, on day 4 after viral inoculation the HW and HW/BW ratio in Can-late and Can-early mice were significantly decreased compared with those in Vehicle and on day 7 was lower than those in Vehicle. However, the HW and HW/BW ratio between Can-late and Can-early groups on day 0, day 4 and day 7 were not significantly different.

3.2. Pathological findings

Myocardial necrosis and inflammation cell infiltration were found in EMC virus-inoculated KKAy mice sacrificed on day 4 and day 7 (Fig. 1). Histological evaluation of hearts showed that treatment with candesartan significantly reduced the severity of the disease, associated with lower the histopathologic scores for myocardial necrosis and mononuclear cell infiltration than vehicle on day 4 and day 7 ($P<0.05$, Table 2). There was no significant difference in histological scores between Can-early and Can-late groups.

The myocardial fiber diameters of the left ventricle were significantly reduced in the Can-early compared with those in Vehicle on days 0, 4 and 7 (Table 2). Those in Can-late

Table 2
Effects of candesartan on histological scores and myocardial fiber diameter in KKAy mice with acute viral myocarditis

Groups	N	Pathologic scores			Myofiber diameter
		Necrosis	Calcification	Inflammation	(μm)
Day 0					
Vehicle	4	ND	ND	ND	12.6±1.2
Can-early	4	ND	ND	ND	11.0±1.3*†
Can-late	4	ND	ND	ND	12.3±1.5
Day 4					
Vehicle	4	0.3±0.1	0.4±0.2	0.4±0.2	12.9±1.5
Can-early	4	0.2±0.1	0.2±0.1	0.2±0.2	11.3±1.4*
Can-late	4	0.2±0.2	0.2±0.1	0.3±0.2	13.3±1.7
Day 7					
Vehicle	4	1.9±0.4	0.4±0.2	1.4±0.5	14.8±2.4†
Can-early	4	1.1±0.3**	0.3±0.2	0.6±0.3**	12.2±1.8**
Can-late	4	1.3±0.3*	0.3±0.2	0.8±0.3*	13.6±1.7*

On days 4 and 7 after viral inoculation, the histopathologic scores for myocardial and cellular infiltration were significantly lower in candesartan treated mice than those in Vehicle. ND; not detected, *$P<0.01$, **$P<0.001$ vs. Vehicle on the same day, †$P<0.01$ vs. Vehicle on day 0 in myofiber diameter.

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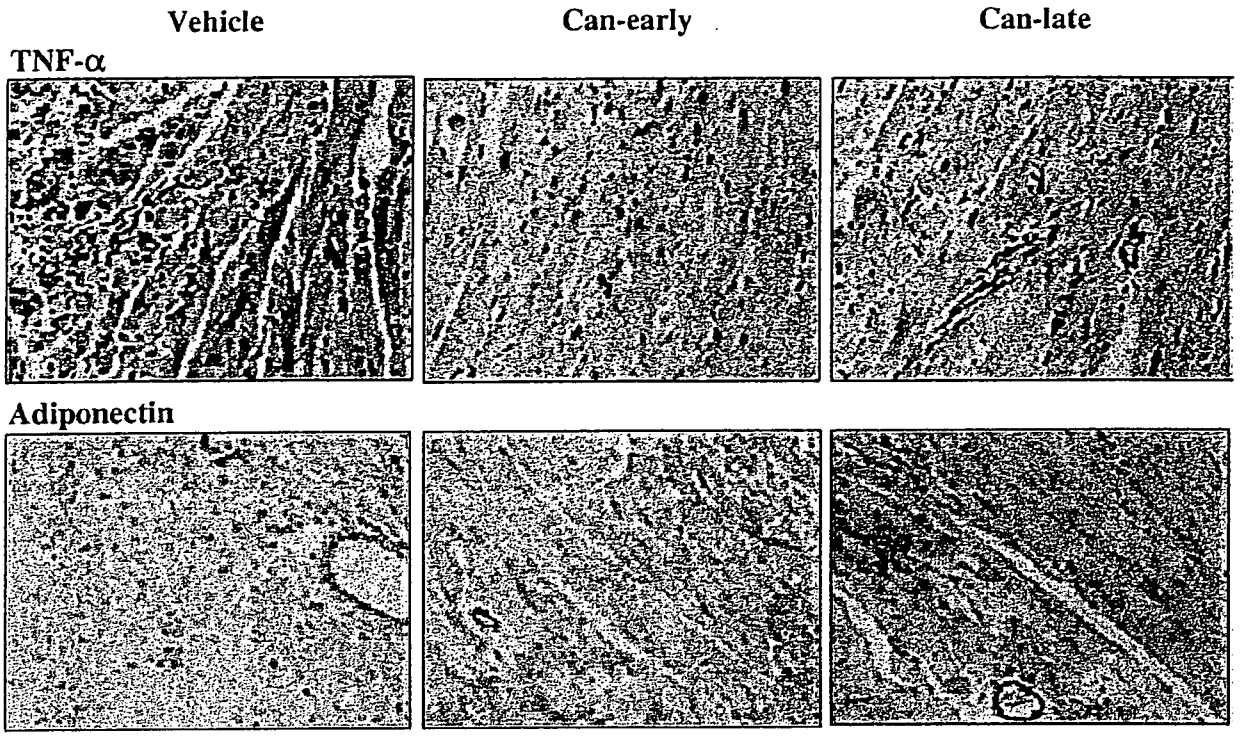

Fig. 2. Immunohistochemical findings. Localized expression of TNF-α and adiponectin in the heart on day 7 was shown. Injured myocytes were positively expressed by ant-murine TNF-α antibody in Vehicle on day 7, and endothelial cells and myofiber were positively expressed in Can-early and Can-late groups.

were significantly lower than those with vehicle on day 7. The myocardial fiber diameters between Can-early and Can-late mice were not significantly different on day 4 and 7.

3.3. Immunohistochemical findings

Cardiac expression of adiponectin protein was positive both in Can-early and in Can-late on day 7 but that was slightly positive in Vehicle group (Fig. 2). Cardiac TNF-α strongly positive in Vehicle but that was weakly positive in Can-early and Can-late on day 7 (Fig. 2). On day 0, cardiac adiponectin was positive but TNF-α was negative in Vehicle and in Can-early and Can-late (data not shown).

3.4. Blood glucose and serum adiponectin levels

Levels of blood glucose in the Can-early were slightly lower than in vehicle, but there was no statistical difference.

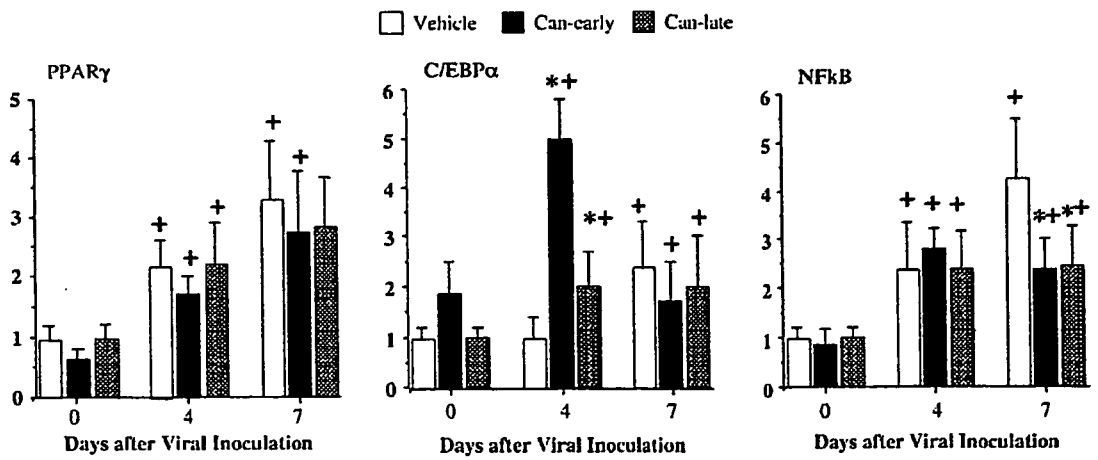

Fig. 3. Comparative expression of cardiac PPAR-γ, C/EBPα and NF-κB mRNA by RT-PCR. The expression levels of PPAR-γ mRNA in the hearts were not statistically different among three groups, however, those of C/EBPα was significantly higher in Can-early in comparison with Vehicle on day 4 (*$P<0.05$, $n=4$ of each). Cardiac NF-κB was significantly lower in Can-early and in Can-late in comparison with Vehicle on day 7 (*$P<0.05$, $n=4$ of each). Cardiac PPAR-γ, C/EBPα and NF-κB were significantly increased in Can-early, Can-late and Vehicle on days 4 and 7 compared with Vehicle on day 0 ($^{\dagger}P<0.05$, $n=4$ of each).

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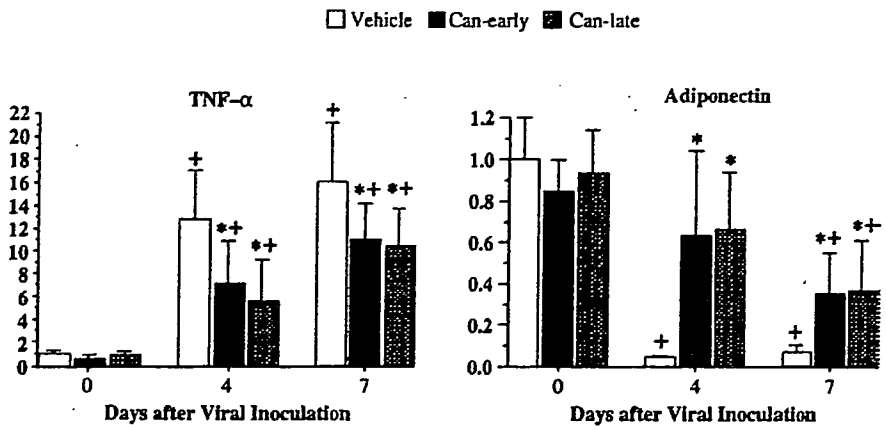

Fig. 4. Comparative expression of cardiac TNF-α and adiponectin mRNA by RT-PCR. Cardiac TNF-α mRNA was significantly less expressed in the heart from Can-early and Can-late than that from Vehicle on days 4 and 7 (*$P<0.05$, $n=4$ of each). The expression levels of adiponectin mRNA were significantly higher in Can-early and Can-late in comparison with Vehicle on days 4 and 7 (*$P<0.05$, $n=4$ of each). Cardiac TNF-α was significantly increased in Can-early, Can-late and Vehicle on days 4 and 7 compared with Vehicle on day 0 ($^{\dagger}P<0.05$, $n=4$ of each). Cardiac adiponectin was significantly decreased in Vehicle on days 4 and 7 compared with Vehicle on day 0 ($^{\dagger}P<0.05$, $n=4$ of each).

on day 0, day 4 and day 7. Serum adiponectin levels were significantly increased in Can-early on day 4 and day 7 compared with those in the vehicle ($P<0.05$, Table 1). Those in Can-late were also significantly increased on day 7 compared with in Vehicle. And there was no difference between Can-early and Can-late groups.

3.5. Myocardial PPAR-γ, C/EBPα, NF-κB, TNF-α and adiponectin mRNA expression

As shown in Fig. 3, cardiac expression of PPAR-γ significantly increased after viral inoculation in either groups in comparison with on day 0 (Fig. 3), that was not significantly altered on days 4 and 7 among 3 groups. Cardiac C/EBPα not increased in Vehicle on day 4, but in the Can-early group, cardiac C/EBPα significantly higher than that in Vehicle. Cardiac NF-κB was increased in Vehicle on days 4 and 7 in comparison with that on day 0. Myocardial NF-κB was significantly reduced in both of Can-early and Can-late groups on day 7. Myocardial TNF-α was significantly reduced in Can-early and Can-late groups compared with Vehicle on day 4 and day 7. Myocardial adiponectin mRNA levels were significantly higher in Can-early and Can-late in days 4 and 7 in comparison with Vehicle (Fig. 4). However there was no significant difference between Can-early and Can-late groups in cardiac NF-κB, TNF-α and adiponectin mRNA on days 4 and 7.

4. Discussion

This study showed that treatment with an AT1-receptor blocker, candesartan significantly reduced myocardial necrosis and cellular infiltration in obese KKAy mice with acute viral myocarditis. Candesartan also induced expression of cardiac adiponectin mRNA via the induction of transcriptional factor C/EBPα mRNA and reduction of NF-κB and TNF-α mRNA.

Obesity is a risk factor of diabetes, metabolic syndrome and different cardiovascular disease, and becoming a public health issue throughout the world. Serum adiponectin is reduced in states of type II diabetic and obese patients [12,19], obese mice models [20] and is further decreased in patients with cardiovascular disease [21]. Adiponectin, that was once thought to be secreted by adipose tissue only [12], recent study show that adiponectin is not adipocyte specific but local production of adiponectin by cardiomyocytes and suggests that the local production of this hormone by cardiomyocytes could be involved in the regulation of cardiac metabolism and function [15]. Our previous study showed that adiponectin was expressed in injured myocytes by autopsy cases [13]. Adiponectin has been reported to play a role in myocardial remodelling after ischemic injury [22]. As agreement with previous studies, we found that the obese mice had lower levels of adiponectin, and further decreased on day 4 and 7 after virus inoculation. These results suggest that hypoadiponectinemia is related to myocardial injury in obese mice with acute viral myocarditis. The AT1-receptor blocker reduced cardiac fibrosis, inflammation and increased adiponectin concentrations [15,23,24]. In our study, administration with candesartan significantly increased adiponectin concentrations and decreased severity of myocardial injury associated with the attenuation of cardiac hypertrophy and inflammation in obese mice with acute viral myocarditis.

C/EBPα transcription factors are expressed in a number of tissues and are involved in the regulation of several biological processes, including control of energy metabolism [25]. Importantly, C/EBPα is known to be a key regulator for adiponectin gene transcription [26] and plays a role in the expression of adipocyte-specific genes [27], suggesting that this transcription factor may regulate the expression of genes related to energy metabolism in myocyte. PPAR-γ regulates not only the expression of genes involved in fatty acid synthesis, oxidation, and storage, but also participates in the

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molecular mechanism of altered metabolic homeostasis, such as type II diabetes or obesity [28,29]. This study showed induced expression of C/EBPα but no alternation of PPAR-γ in candesartan treated mice. Further study will be needed for the different responses of these adiponectin transcription factors.

NF-κB is activated by several factors, which increase the inflammatory response and this activation, leads to the expression of several genes such as cytokines, and may play an important role in cardiovascular diseases [30]. NF-κB inhibitor suppressed the expressions of cardiac TNF-α in vivo and vitro and prevented the development of myocarditis [30]. It was also reported that adiponectin reduced the activation of NF-κB [31]. The AT1-receptor blocker significantly attenuated expression of TNF-α and activation of NF-κB [8]. Our study showed candesartan reduced cardiac NF-κB as well as TNF-α and increased the expression of cardiac adiponectin. Our previous data showed lower NF-κB mRNA in the heart of food-restricted mice than in obese mice [19]. Decreased expression of cardiac NF-κB could reduce viral replication and inflammation in the heart [32]. Recent report showed that NF-κB was required for the hypertrophic response of cardiomyocytes in vitro [33]. Indeed, our data showed that candesartan decreased myocardiac necrosis and lowered myofiber diameter with downregulation of NF-κB expression.

Excess amount of cytokine induced by inflammatory stimuli contributes to the progression of myocardial damage in myocarditis [5,19,34]. The levels of circulating TNF-α are elevated in patients [34] and mice with myocarditis associated with extensive myocardial necrosis and inflammation [35]. TNF-α contributes to the onset of acute myocardial rupture and chronic left ventricle dysfunction by inducing exuberant local inflammatory response, matrix and collagen degradation and apoptosis [36]. These reports demonstrated that adiponectin and TNF-α suppress each other's production and also antagonize each other's action in their target tissues [37]. In our study, administration of candesartan significantly increased the circulating adiponectin and myocardial adiponectin mRNA with conversely decreased cardiac TNF-α mRNA. Increased myocardial adiponectin may prevent the expression of myocardial TNF-α mRNA, contributing to the prevention of accelerated myocarditis caused by overproduction of TNF-α in the heart.

To the best of our knowledge, the present study is the first demonstration regarding the role of candesartan, an AT1-receptor antagonist, on acute virus myocarditis and cardiac adiponectin mRNA level under obesity and diabetes. The cardioprotective effect of candesartan may be due to the modulation of cardiac adiponectin mRNA and the suppression of TNF-α mRNA via its regulation of the C/EBPα and NF-κB transcription factors.

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Original Article

Migraine Is Associated with Enhanced Arterial Stiffness

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Migraine is a common subtype of headache. Epidemiological studies have revealed that migraine could be an independent risk factor for ischemic stroke even in elderly subjects. Arterial stiffness is one of the major pathophysiological bases of stroke. In the present study, we cross-sectionally investigated the possible relationship between migraine and arterial stiffness in community-dwelling subjects. The study subjects were independently recruited from two sources (Group A, n=134, 68±5 years; Group B, n=138, 68±7 years). Augmentation index (AI), the ratio of augmented pressure by the reflection pressure wave to the pulse pressure, was obtained from the radial arterial waveform as an index of arterial stiffness. Brachial blood pressure was also measured simultaneously. Migraine was diagnosed using a previously validated questionnaire. The prevalence of migraine was 5.2% (Group A) and 16.7% (Group B). Subjects with migraine had higher radial AI in both Group A (migraine, 101±15%; other headache, 88±12%; no headache, 86±12%, p=0.003) and Group B (95±11%, 90±11%, 91±14%, p=0.058). Multiple linear regression analysis revealed that migraine was an independent determinant of AI (β=0.154, p=0.002) after adjustment for other confounding factors: age (β=–0.024, p=0.654); sex (β=0.141, p=0.069); body height (β=–0.215, p=0.005); systolic blood pressure (β=0.174, p=0.001); medication for hypertension, hyperlipidemia, and diabetes mellitus (β=–0.014, p=0.787); and heart rate (β=–0.539, p<0.001). In a separate analysis by sex, migraine was also a significant determinant for AI (male, β=0.246, p=0.019; female, β=0.159, p=0.008). Migraine in the elderly could be a clinical manifestation of enhanced arterial stiffness. (*Hypertens Res* 2007; 30: 577–583)

***Key Words*: migraine, arterial stiffness, elderly, augmentation index**

Introduction

Migraine is a common subtype of headache with specific characteristics including unilaterality, throbbing pain, photophobia or phonophobia, and nausea or vomiting (*1*). Several large-scale epidemiological studies have revealed that the prevalence of migraine ranges from 6 to 13% in the general population (*2–6*). The prevalence is approximately three to four times higher in females, and is highest among women aged in their thirties and forties (*7, 8*). Migraine is also commonly observed in the elderly (*8, 9*).

Headaches, in particular migraine, are known to be an independent risk factor for ischemic stroke (*4–6, 10–16*). The association between migraine and stroke is more prominent in young women, particularly in those taking oral contraceptives (*11–14*). However, in the middle-aged to elderly of either sex, the association is controversial. Several epidemiological studies have indicated that severe headache and migraine should be considered risk factors for future stroke prior to the age of

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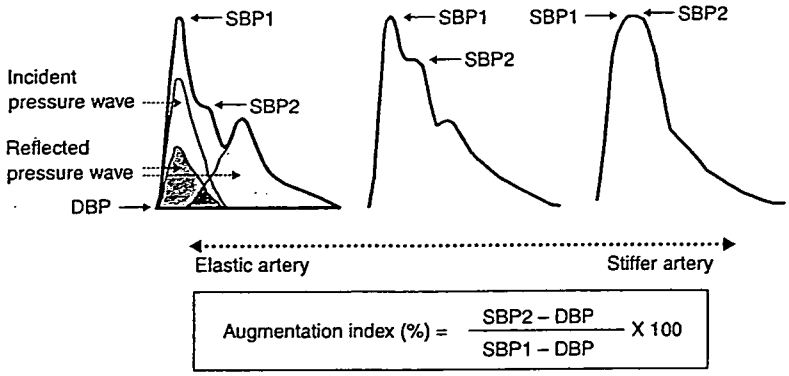

Fig. 1. *Tracing of radial arterial waveform. The radial augmentation index was calculated as follows: (second peak of SBP [SBP2] – DBP)/(first peak of SBP [SBP1] – DBP) × 100 (%). SBP, systolic blood pressure; DBP, diastolic blood pressure.*

70 (*5, 15*). In contrast, no significant association was found in a case-control study with elderly subjects aged 60 or older (*17*).

The pathophysiological mechanism by which migraine may lead to ischemic stroke is unclear. Kruit *et al.* (*18*) showed that a combination of migraine attack–related hypoperfusion and embolism could be an underlying mechanism of infarction frequently observed in the posterior circulation territory in migraine patients. However, during a migraine attack, vascular changes are not limited to the cranial circulation. Iversen *et al.* (*19*) reported that migraine attacks are accompanied by generalized peripheral vasoconstriction. Furthermore, migraine patients have displayed increased diameter and/or decreased distensibility of cranial and peripheral blood vessels even in the interictal period (*20*). These observations indicate the importance of vascular properties in the link between migraine and ischemic stroke.

Recently, several parameters have been introduced to assess vascular stiffness (*21*). Augmentation index (AI) is a parameter of arterial stiffness that can be obtained from the central arterial waveform as the ratio of augmentation pressure by the reflection pressure wave to the pulse pressure. It has been reported that central AI is closely related to several risk factors for atherosclerosis (*22*) and future cardiovascular events (*23, 24*). AI can also be obtained from the radial arterial waveform (*25*). Since radial AI is closely associated with aortic AI (*25*), radial AI itself could provide information on vascular properties.

In the present study, we measured radial AI as an index of arterial stiffness to investigate the possible association between migraine and enhanced arterial stiffness in community-dwelling elderly subjects.

Methods

Study Subjects

The study subjects were independently recruited from two sources: attendees of a public exercise seminar held by the city of Matsuyama (n=134, Group A), the largest city in Japan's Ehime Prefecture; and participants of a medical check-up program at Ehime University Hospital (n=138, Group B). These cross-sectional investigations were carried out as parts of the Shimanami Health Promoting Program (J-SHIPP study), a longitudinal study evaluating factors related to cardiovascular disease, dementia, and death (*26–29*). The exercise seminar was held twice a week for a 9-month period (from July 2002 to March 2003). Participants in the seminar (n=208) were recruited from among the general residents of Matsuyama City. Measurement of blood pressure (BP) and AI was carried out before exercise training during July 2002 to January 2003. The number of participants who gave written informed consent and completed all measurements was 134 (67.0%). None of these subjects had a known history or symptoms of cerebrovascular diseases. The medical check-up, called the "anti-aging dock," which is designed specifically for evaluating aging-related disorders, was also carried out among general residents of Ehime Prefecture. Among the participants from March to August 2006 (n=185), those who provided informed consent (n=161, 87.0%) and were free from any history or symptoms of cerebrovascular disease (n=138, 74.6%) were enrolled in this analysis. The series of studies was approved by the ethics committee of Ehime University School of Medicine, and all participants gave written informed consent to participate in the procedure.

Measurement of BP and AI

Brachial BP and radial arterial waveform were measured simultaneously, and radial AI was calculated from the waveform using a semi-automatic waveform analyzer (HEM-9000AI, OMRON HEALTHCARE Co., Ltd., Kyoto, Japan). In brief, the arterial waveform was non-invasively obtained from the left radial artery by tonometric tracing. The tonometric sensor consisted of 40 arrayed microtransducers within 8 mm, and the most appropriate one was automatically selected

Table 1. Baseline Characteristics of Study Subjects

	Study group		*p*
	A (134)	B (138)	
Age (years)	68±5	68±7	0.901
Sex (male/female)	16/118	56/82	<0.001
Body height (cm)	154±7	158±9	<0.001
Body weight (kg)	54±9	58±12	0.001
Systolic blood pressure (mmHg)	139±20	144±19	0.038
Diastolic blood pressure (mmHg)	78±11	81±12	0.031
Medication (%)			
Hypertension	22.4	31.2	0.132
Hyperlipidemia	9.7	20.3	0.017
Diabetes mellitus	2.2	0.0	0.118
Heart rate (beats/min)	73±10	68±12	<0.001
Radial augmentation index (%)	88±13	91±12	0.021
History of cardiovascular disease (*n*)	2	9	0.060

Cardiovascular disease consisted of 1 case of myocardial infarction, 8 cases of angina pectoris and 2 cases of atrial fibrillation.

Table 2. Prevalence and Frequency of Headache

	Study group	
	A (134)	B (138)
Subjects with headache (*n*)		
Migraine	7	23
Other headache	40	62
Response to ID Migraine questionnaire items (*n*)		
Nausea or vomiting	7	18
Disability of working, study or other activity	0	3
Photophobia	1	5
Frequency of headache (*n*)		
Several time a year	29	26
1 or 2 times a month	14	36
1 or 2 times a week	2	14
3 or 4 times a week	2	4
Daily or nearly daily	0	5

for the optimal observation. The sensor head's hold-down pressure was also automatically adjusted for each subject. Waveforms were measured for 30 s and digitized at 500 Hz. Brachial BP was simultaneously measured in the right upper arm by the cuff-oscillometric method. All measurements were carried out with subjects in the sitting position after at least 5 min of rest. The exercise seminar employed a prototype analyzer that is identical to HEM-9000AI except that it uses a laptop computer for the acquisition and analysis of waveforms. Brachial BP was measured using another cuff-oscillometric device (HEM-907, OMRON HEALTHCARE Co., Ltd.).

Radial AI was calculated as the ratio of late systolic pressure to pulse pressure: (late systolic BP [SBP2] – diastolic BP [DBP])/(systolic BP [SBP1] – DBP) ×100 (%) (Fig. 1), which was automatically calculated using a fourth-order differential equation for radial arterial waveform (HEM-9000AI) (*30*). The intra- and inter-measurement variability of radial AI was 4.6% and 3.4%, respectively. A nomogram of radial AI and its correlation with aortic AI has been described elsewhere (*25*).

Evaluation of Migraine

Episodes of migraine were evaluated using ID Migraine, a self-administered questionnaire (*31*). This questionnaire consists of three questions on disability (How many days did your headache limit your ability to work, study, or do what you needed to do?), nausea (You felt nauseated or vomiting during migraine attack), and photophobia (Light bothered you during migraine attack [a lot more than when you don't have headaches]). The validity and reproducibility of ID Migraine has been confirmed previously using the International Headache Society–based migraine diagnosis as a reference (*31*). Cross-validation over sex, age, presence of other comorbid types of headache, or previous diagnosis of migraine has also been performed (*31*). Subjects with any of these three complaints were considered to have migraine.

Statistical Analysis

Values are expressed as means±SD unless otherwise specified. All statistical analyses were performed using the SPSS software package (SPSS Inc., Chicago, USA). The differences among categories were analyzed using one-way analysis of variance (ANOVA). Differences in prevalence or frequency were analyzed by χ^2 test. Factors independently associated with AI were assessed using multiple linear regres-

Table 3. Clinical Characteristics of Subjects with Migraine

	Migraine	Other headache	No headache	*p*
Study group A				
n	7	40	87	
Age (years)	67±3	68±5	68±6	0.559
Sex (female %)	100.0	87.5	87.4	0.402
Body height (cm)	151±4	154±7	154±7	0.239
Body weight (kg)	51±7	54±7	55±9	0.372
Systolic blood pressure (mmHg)	129±10	140±20	139±21	0.171
Medication (%)	14.3	40.0	26.4	0.674
Heart rate (beats/min)	72±10	73±11	74±10	0.637
Augmentation index (%)	101±15	88±12	86±12	0.003
Study group B				
n	23	62	53	
Age (years)	66±10	68±7	69±6	0.180
Sex (female %)	91.3	56.5	49.1	<0.001
Body height (cm)	157±6	157±8	159±9	0.414
Body weight (kg)	55±7	57±12	61±12	0.157
Systolic blood pressure (mmHg)	144±23	143±19	145±19	0.916
Medication (%)	26.1	41.9	47.2	0.163
Heart rate (beats/min)	68±12	70±11	66±12	0.980
Augmentation index (%)	95±11	90±11	91±14	0.058
Combined				
n	30	102	140	
Age (years)	66±9	68±6	68±6	0.130
Sex (female %)	93.3	68.6	72.9	0.005
Body height (cm)	155±6	156±8	156±8	0.661
Body weight (kg)	54±8	56±11	57±11	0.268
Systolic blood pressure (mmHg)	141±21	142±19	141±20	0.823
Medication (%)	23.3	41.2	34.3	0.160
Heart rate (beats/min)	69±12	72±11	71±11	0.390
Augmentation index (%)	97±12	89±11	88±13	<0.001

Values are mean±SD. Statistical significance was assessed between subjects with and without migraine. Total number of subjects under treatment of hypertension, hyperlipidemia, and diabetes mellitus is described as frequency.

sion analysis. Analysis of covariance was used to obtain adjusted AI and its group differences. A p value of less than 0.05 was considered statistically significant.

Results

Baseline anthropometric and clinical characteristics of the two study groups are shown in Table 1. Two subjects in Group A had a history of myocardial infarction and angina pectoris. Nine subjects in Group B had a history of several cardiovascular diseases; 7 with angina pectoris and 2 with atrial fibrillation. There were no significant differences in age or use of antihypertensive medication. The proportion of male subjects was higher in Group B. Radial AI was significantly higher in Group B.

The prevalence of migraine is summarized in Table 2. In Group A, 47 subjects (35.1%) had headaches, and 7 (5.2%) were diagnosed as having migraine by the ID Migraine questionnaire. The prevalence of headaches (61.6%, $p<0.001$) and the proportion of migraine subjects (16.7%, $p=0.003$) were significantly higher in Group B. Among the three items of the ID Migraine questionnaire, nausea was the most frequently observed complaint. The number of subjects reporting disability in daily work was quite small, which may reflect Japanese cultural traits. The majority of migraine subjects (79.5%) had headache attacks less than once or twice a month. This frequency was not different between the subtypes of headache (83.4% for migraine, 78.4% for other).

Table 3 shows the anthropometric and clinical characteristics of the subjects with migraine. In both study groups, migraine subjects had higher AI, while other major confounding factors including age, body height, BP, and heart rate (HR) were not different. Combined analysis further revealed a higher AI in subjects with migraine. To further clarify the

Table 4. Multiple Liner Regression Analysis for Augmentation Index

	Unstandardized coefficient (95% CI)	Standardized coefficient	*p*
Age (years)	−0.046 (−0.245 to 0.154)	−0.024	0.654
Sex (female)	3.977 (−0.319 to 8.272)	0.141	0.069
Body height (cm)	−0.334 (−0.566 to −0.102)	−0.215	0.005
Systolic blood pressure (mmHg)	0.1109 (0.046 to 0.173)	0.174	0.001
Medications	−0.352 (−2.923 to 2.218)	−0.014	0.787
Heart rate (beats/min)	−0.597 (−0.704 to −0.490)	−0.539	<0.001
Migraine (yes)	6.095 (2.239 to 9951)	0.154	0.002

CI, confidence interval.

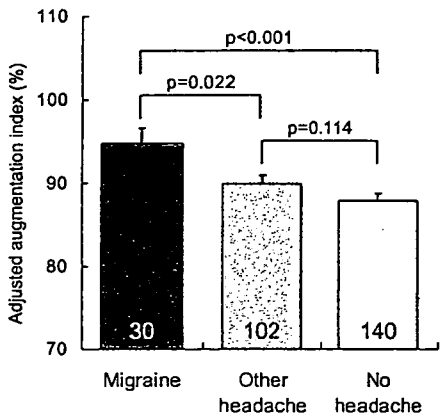

Fig. 2. *Migraine and adjusted augmentation index. Adjusted augmentation indices are represented as means±SEM. Adjusted confounding factors are age, sex, body height, systolic blood pressure, heart rate, and medications. The differences among groups were assessed by analysis of covariance (ANCOVA). The number of subjects in each group is shown in the column.*

relationship between migraine and AI, multiple linear regression analysis was carried out (Table 4). Short stature could be an independent determinant for AI by affecting the arrival time of wave reflection. It is also known that higher HR reduces AI by shortening the cardiac ejection time. However, migraine was an independent determinant of AI after adjustment for these confounding factors. Adjusted AI and its group differences are illustrated in Fig. 2.

To eliminate the effect of medications, multiple linear regression analysis was carried out again in the subjects not taking any medications (n=175). The relationship between AI and migraine remained statistically significant (β=0.204, p=0.002) along with body height (β=−0.263, p=0.011) and HR (β=−0.44, p<0.001). After further exclusion of 3 subjects with a history of cardiac disease (n=172), migraine was still a significant determinant for AI (β=0.202, p=0.002). Furthermore, regression analysis was separately performed in each sex because of the higher prevalence of migraine in female subjects (Table 3). The presence of migraine was an independent determinant of AI in both male (β=0.246, p=0.009) and female (β=0.159, p=0.008) subjects.

Discussion

The present study revealed that subjects with migraine had greater arterial stiffness than migraine-free subjects in the two study groups independently recruited from community residents. This association was independent of other confounding factors including age, sex, body height, BP, and HR. These observations suggest the possibility that migraine could be a manifestation of arterial stiffness in the elderly.

Serotonin (5-HT) is a key mediator of migraine (*32*). It is known that stimulation of the 5-HT1 receptor leads to cranial vasoconstriction, peripheral neuronal inhibition, and inhibition of transmission through second-order neurons of the trigeminocervical complex, and thus alleviates migraine and associated symptoms (*33*). Most studies have shown that the systemic 5-HT content of platelets in migraine patients is lower than that in healthy controls (*34*). It has also been reported that plasma norepinephrine levels are significantly lower in migraine subjects (*35*). Since sympathetic nervous activity regulates AI by changing arterial tone, the observations in this study may conflict with the autonomic and humoral properties underlying migraine. However, arterial stiffness is also determined by vessel wall structure. de Hoon *et al.* (*20*) have shown higher intima-media thickness in the brachial artery in middle-aged migraine patients. Elevated serum elastase activity has also been reported in migraine subjects (*36*). Abnormalities in the extracellular matrix are a possible explanation for the relationship between migraine and enhanced AI. Measurement of AI during migraine attacks may lead to a better understanding of the observed relationship.

Even in elderly subjects, migraine could be an independent risk factor for ischemic stroke. However, the relationship was weaker in the elderly than in young subjects despite the higher incidence of stroke in the former (*5*). Enhanced arterial stiffness is known to be a potent risk factor for stroke in the