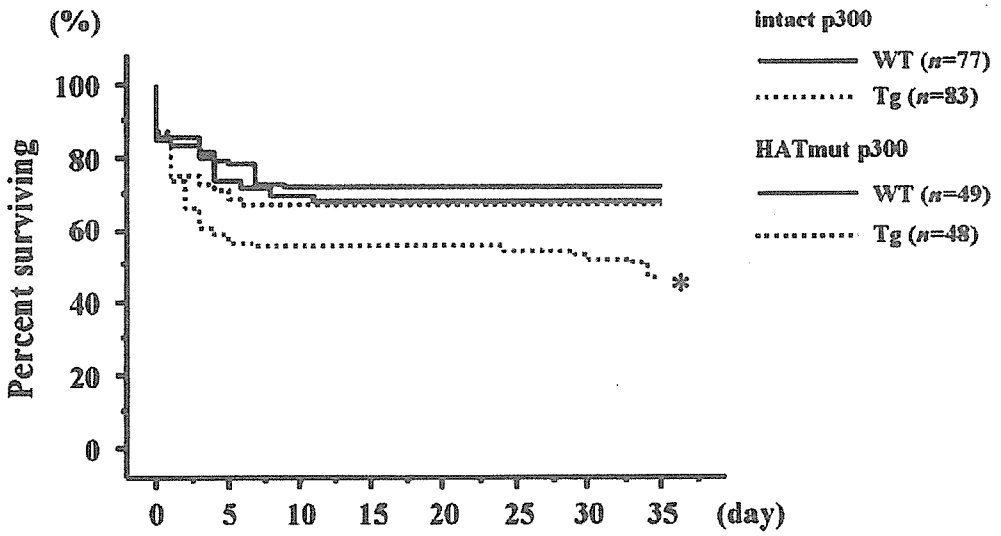
A



* $p < 0.05$ vs WT and HATmut p300 Tg

B

	intact p300				HATmut p300			
	WT		Tg		WT		Tg	
MI	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
	n=15	n=21	n=17	n=18	n=16	n=23	n=21	n=16
HR (bpm)	443 ± 47	456 ± 90	441 ± 30	425 ± 39	468 ± 49	476 ± 63	475 ± 37	460 ± 42
systole BP (mmHG)	118 ± 10	117 ± 10	116 ± 9	114 ± 10	124 ± 13	123 ± 13	122 ± 9	114 ±12
diastole BP (mmHG)	51 ± 9	52 ± 10	53 ± 14	55 ± 11	60 ± 11	60 ± 11	58 ± 12	53 ±8
LVEDD (mm)	3.57 ± 0.22	4.43 ± 0.59 †	3.74 ± 0.25	5.41 ± 0.51 *¶	3.41 ± 0.11	4.31 ± 0.20 †	3.63 ± 0.23	4.39 ± 0.37 #
LVESD (mm)	1.90 ± 0.26	3.20 ± 0.51 †	2.03 ± 0.20	4.55 ± 0.68 *¶	1.86 ± 0.27	3.16 ± 0.27 †	1.98 ± 0.24	3.21 ± 0.47 #
FS (%)	46.9 ± 4.50	28.0 ± 7.60 †	45.6 ± 3.40	16.3 ± 6.30 *¶	45.6 ± 7.10	26.6 ± 4.80 †	45.5 ± 5.50	27.1 ± 5.90 #

†$p<0.05$ versus corresponding WT MI(-) *$p<0.05$ versus intact p300-Tg MI(-)
¶ $p<0.05$ versus HATmut p300-Tg MI(+) #$p<0.05$ versus HATmut p300-Tg MI(-)

C

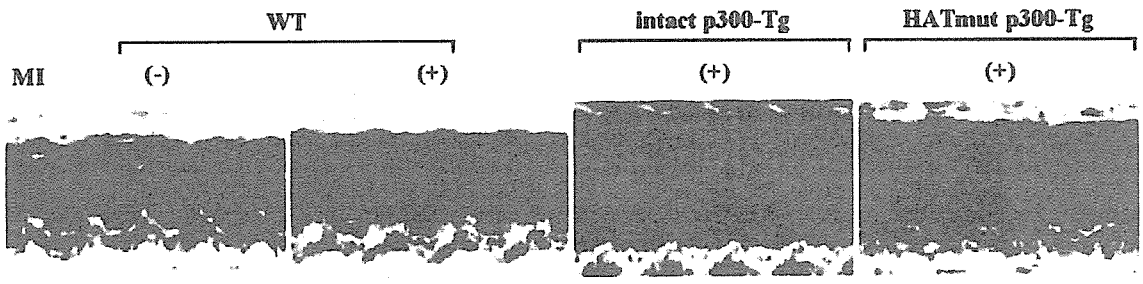


Figure 4. Survival rates and echocardiography data after MI. At the age of 12 weeks, intact p300-Tg mice, their corresponding WT mice, HATmut p300-Tg mice, and their corresponding WT mice were subjected to sham or MI operation. A, Kaplan-Meier survival curves of MI-operated mice. B, Echocardiographic data at 5 weeks after sham or MI operation. HR indicates heart rate; BP, blood pressure; LVEDD, left ventricular chamber diameter in end-diastole; LVESD, left ventricular chamber diameter in end-systole; FS, fractional shortening. C, Representative photographs of M-mode images.

(Figure 8A). GATA-4/DNA binding in the heart was increased after MI in each kind of mouse. In the MI groups, however, the intensity of GATA-4/DNA binding was significantly higher in intact p300-Tg than in WT mice but was similar between HATmut p300-Tg and WT mice (Figure 8, B and C). In contrast, cardiac Sp-1 binding activities were similar among all kinds of mice (Figure 8D). Finally, we measured LV levels of ET-1, a downstream target of the

A

	intact p300				HATmut p300			
	WT		Tg		WT		Tg	
MI	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
	n=7	n=3	n=5	n=5	n=3	n=3	n=3	n=3
HR (min⁻¹)	392 ± 11	485 ± 46	392 ± 25	374 ± 11	425 ± 25	438 ± 36	429 ± 22	429 ± 19
ESP (mmHg)	96.1 ± 6.1	88.5 ± 14.4	91.2 ± 4.5	83.4 ± 5.3	95.8 ± 6.7	98.0 ± 9.7	101.7 ± 7.4	94.7 ±3.6
EDP (mmHg)	1.8 ± 0.4	4.9 ± 0.5	4.9 ± 0.7	15.3 ± 2.5 ‡*¶	4.1 ± 0.5	7.1 ± 1.4	5.4 ± 2.2	9.4 ±2.5
ESV (μL)	7.5 ± 0.8	27.5 ± 8.1 †	13.5 ± 2.0	94.3 ± 18.8 ‡*¶	8.7 ± 1.3	25.3 ± 3.8†	9.7 ± 3.2	26.3 ± 2.9 #
EDV (μL)	19.2 ± 1.6	36.2 ± 10.7 †	25.7 ± 1.7	98.6 ± 18.4 ‡*¶	23.9 ± 3.2	34.1 ± 4.9	27.6 ± 2.3	32.6 ± 4.6
SI (μL/g)	0.58 ± 0.07	0.42 ± 0.15	0.59 ± 0.11	0.19 ± 0.08 *	0.73 ± 0.13	0.48 ± 0.06	0.82 ± 0.11	0.28 ± 0.08 #
EF (%)	59.8 ± 4.3	24.4 ± 7.1 †	47.1 ± 6.8	4.7 ± 2.4 ‡*¶	63.7 ± 2.4	26.1 ± 2.4 †	65.7 ± 9.6	18.1 ± 4.3 #
CI (μL/min/g)	230 ± 33	156 ± 41	234 ± 53	69 ± 31 *	314 ± 70	179 ± 37	355 ± 62	121 ±39 #
dP/dtmax (mmHg/s)	8388 ± 1324	7049 ± 2231	7266 ± 1029	3612 ± 473	7700 ± 2407	8219 ± 2385	8542 ± 235	6114 ±822
dP/dtmin (mmHg/s)	-5200 ± 737	4644 ± 1124	3691 ± 92	1935 ± 156 ‡*	-5132 ± 1177	-5096 ± 1054	-6309 ± 690	-3700 ± 314
τ (ms)	9.3 ± 0.7	10.6 ± 2.2	14.3 ± 0.6†	24.0 ± 2.9 ‡¶	9.1 ± 1.5	12.4 ± 0.7	9.4 ± 0.9	12.5 ± 0.3
NL Ees (mmHg/μL · 100mg)	16.80 ± 4.57	8.45 ± 2.69 †	11.26 ± 1.60	2.73 ± 1.18 *	12.71 ± 1.05	6.95 ± 0.70 †	12.22 ± 0.39	6.97 ± 0.81 #
NL Eed (mmHg/μL · 100mg)	0.39 ± 0.12	0.66 ± 0.12	0.37 ± 0.07	1.45 ± 0.12 ‡*	0.45 ± 0.21	0.74 ± 0.15	0.25 ± 0.17	1.59 ± 0.47 #
PRSW (mmHg)	70.0 ± 6.3	50.1 ± 7.5 †	62.9 ± 4.6	28.3 ± 5.9 ‡*¶	69.5 ± 4.6	39.5 ± 13.6 †	58.9 ± 11.7	40.5 ±2.4

†$p<0.05$ versus corresponding WT MI(-) ‡$p<0.05$ versus corresponding WT MI(+) *$p<0.05$ versus intact p300-Tg MI(-)
¶ $p<0.05$ versus HATmut p300-Tg MI(+) #$p<0.05$ versus HATmut p300-Tg MI(-)

B

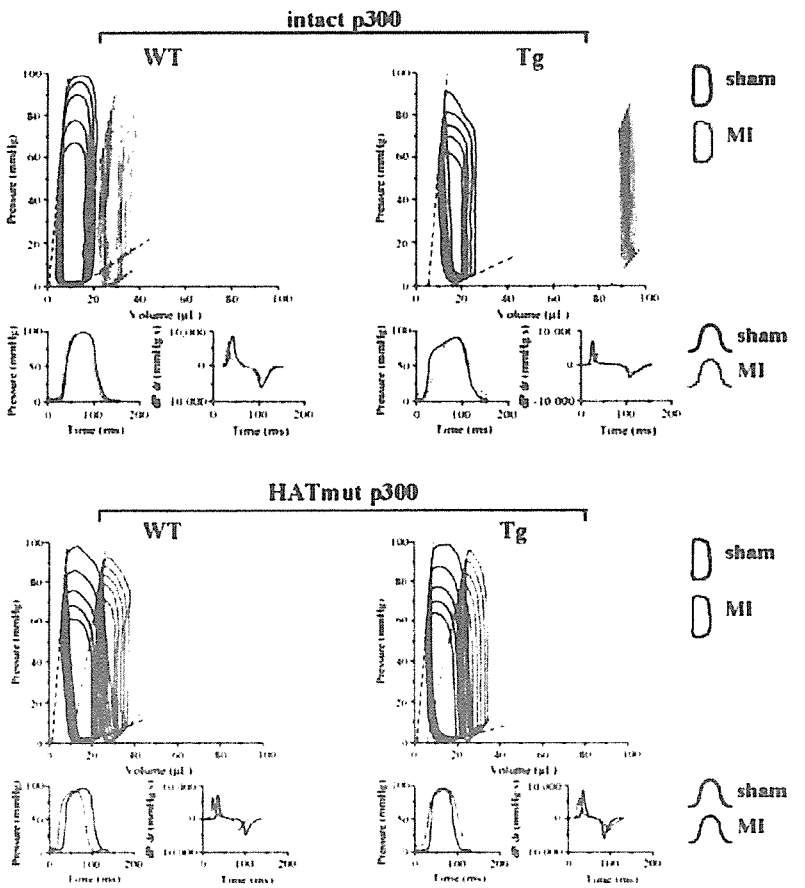


Figure 5. Hemodynamics of Tg and WT mouse hearts after MI. Five weeks after sham or MI operation, hemodynamics of Tg mice and their WT mice were examined as described in the Methods section. A, Values represent mean±SEM of 3 to 7 mice in each group, as indicated. ESP indicates end-systolic pressure; EDP, end-diastolic pressure; ESV, end-systolic volume; EDV, end-diastolic volume; SI, stroke volume index; CI, cardiac index; dP/dtmax, maximum derivative of change in systolic pressure over time; dP/dtmin, minimum derivative of change in diastolic pressure over time; τ, time constant of isovolumic relaxation; NL Ees, normalized end-systolic volume elastance; NL Eed, normalized end-diastolic volume elastance; PRSW, preload recruitable volume work. B, Representative curves of pressure-volume relations, LV pressure, and slope of derivative of change in systolic pressure over time (dP/dt) in sham versus MI.

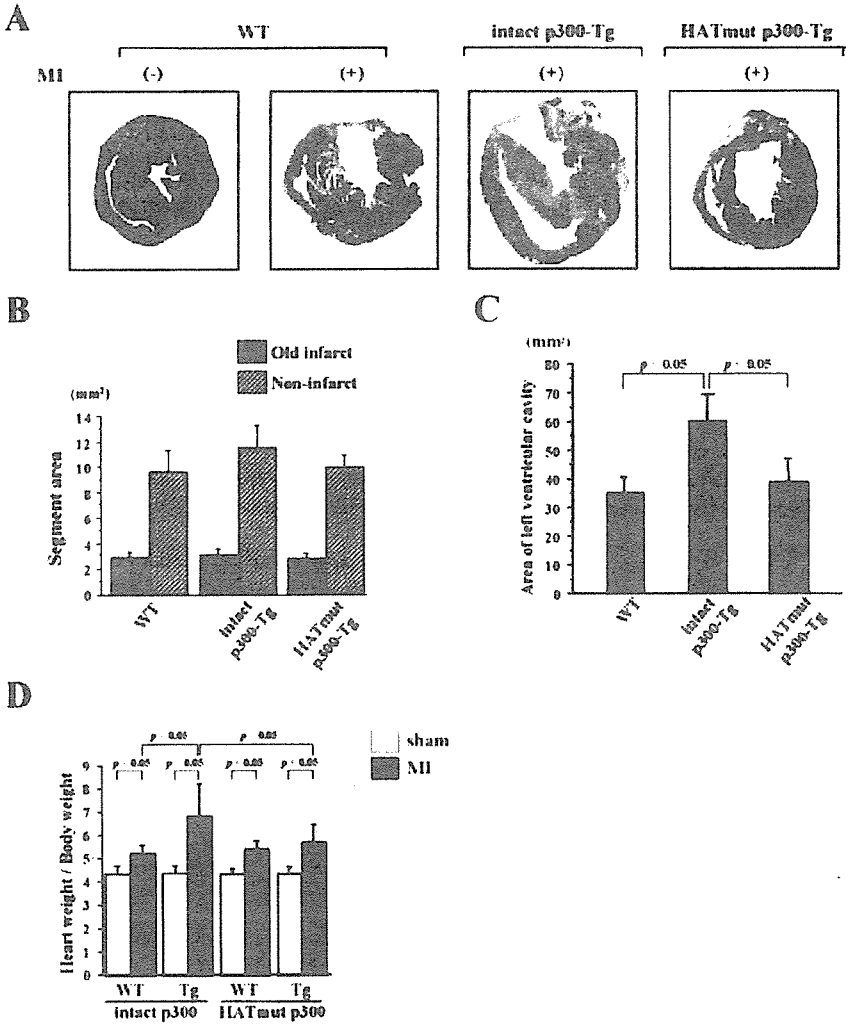


Figure 6. Morphology of Tg and WT mouse hearts after MI. A through C, Transverse sections of mouse hearts at 5 weeks after sham or MI operation were stained with Masson trichrome. A, Representative photographs. B and C, Areas of old infarct and noninfarct and those of LV cavity were measured. Results from 7 animals in each group are expressed as mean±SD. D, Results of heart weight (mg)/body weight (g) ratio from more than 20 animals in each group are expressed as mean±SD.

p300/GATA-4 pathway (Figure 8E). Although LV ET-1 levels in the sham operation groups did not differ among WT, intact p300-, and HATmut p300-Tg mice, the levels were increased after MI in each kind of mouse. Consistent with the findings of EMSAs, the LV ET-1 levels in the MI groups were higher in intact p300-Tg than HATmut p300-Tg mice. These findings suggest that the HAT activity of p300 is involved in the MI-induced increase in the LV ET-1 level as well as GATA-4/DNA binding.

Discussion

The present study investigated the role of p300 HAT activity in LV remodeling after MI in vivo. The HAT domain of p300 has been mapped previously to residues 1284 to 1669.[18] p300 HAT activity is able to acetylate not only histone tails but also transcriptional regulators such as p53 or p300 itself.[11–14] HATmut p300, a mutant we used in the present study, was almost completely defective in histone-p53 acetylation and autoacetylation.[18] In agreement with these findings, HATmut p300 was unable to induce acetylation or DNA binding of GATA-4 in adult mouse hearts as well as in vitro, although acetylation of other components of the transcriptional machinery might be affected in the heart of HATmut p300-Tg. However, HATmut p300 did not act as a transcriptional repressor but had modest ability to enhance GATA-4–dependent transcription. These findings suggest that HATmut p300 acts as a silent mutant and not as a dominant-negative mutant. Three cysteine-histidine–rich domains, which are important in mediating protein-protein interaction, are conserved in HATmut p300. Therefore, the bridging function of HATmut p300 will be conserved in this mutant and might be involved in the modest activation of GATA-4–dependent transcription.

The present study demonstrated that acetylation of GATA-4 in WT hearts increased after MI, concomitant with an increase in expression of endogenous p300. Thus, p300 may be one of the factors that mediate the acetylation during LV remodeling. However, acetylation and DNA binding of GATA-4 in Tg mouse hearts increased after MI even without an increase in the total p300 content. During myocardial cell hypertrophy, activation of mitogen-activated protein kinases induces p300 phosphorylation, which results in enhanced p300 HAT activity.[23,24] Thus, such modification of p300 as well as changes of its quantity might regulate the acetylation of GATA-4 during LV remodeling after MI. It is also possible that other HATs

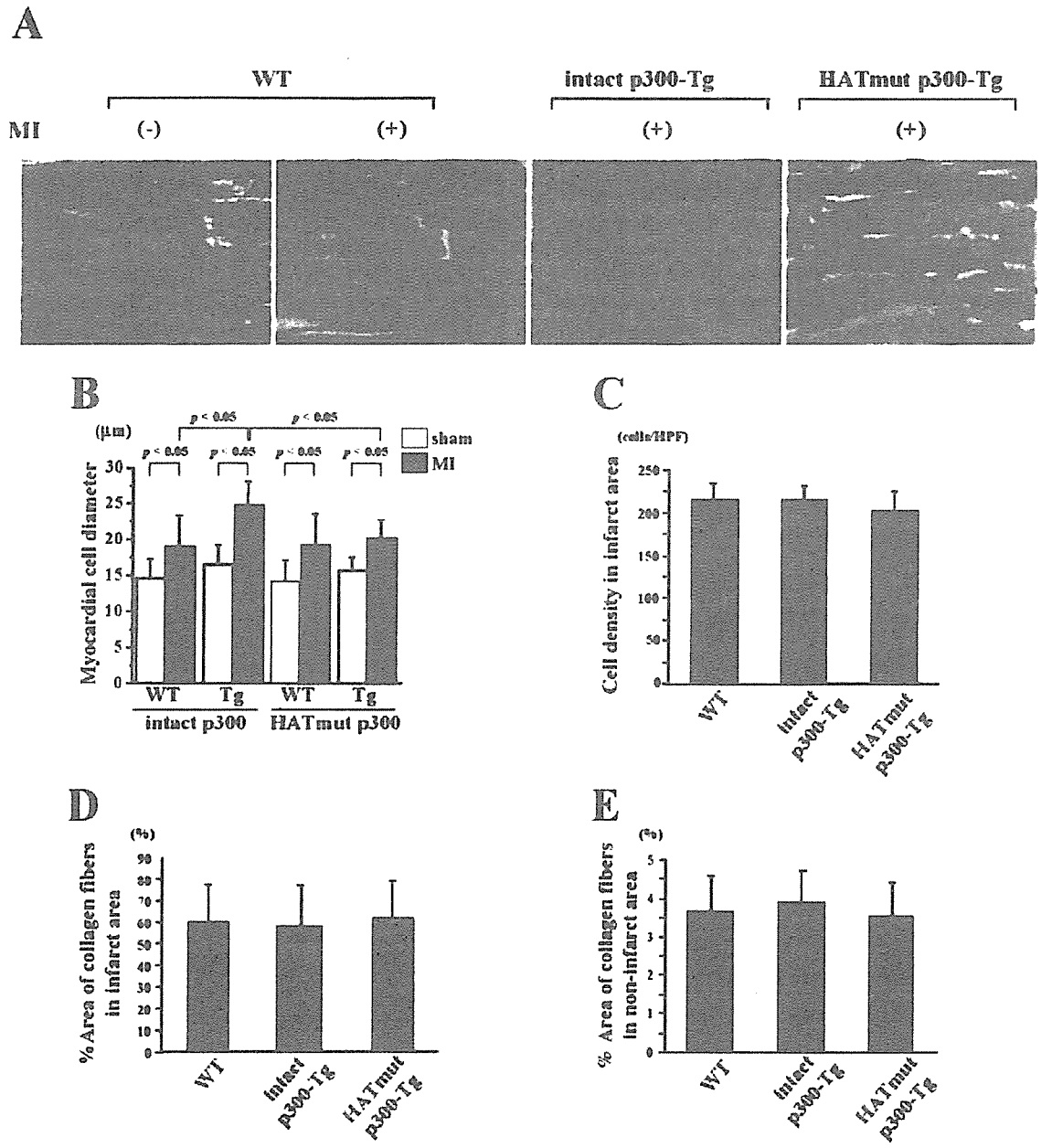


Figure 7. Histological analysis of Tg and WT mouse hearts after MI. A through E, Five weeks after sham or MI operation, histological sections of mouse hearts were subjected to hematoxylin and eosin staining (A through C) or sirius red staining (D and E). A, Representative photographs at magnification ×400. B, The average of 50 individual myocardial cell diameters were calculated for 1 animal, and results from 5 animals in each group are expressed as mean±SD. C, Cell density in infarcted area was measured in each group. D and E, Percentage of areas taken up by collagen fibers in either infarcted (D) or noninfarcted (E) region. Results from 7 animals in each group are expressed as mean±SD (C through E).

such as p300/CBP associating factor (P/CAF) play a role in MI-induced acetylation. In addition, acetylation of nuclear proteins is regulated by histone deacetylases.[25] Therefore, to clarify the precise mechanisms of acetylation during LV remodeling after MI, it would be interesting to examine the association of GATA-4 or myocyte enhancing factor-2 with p300 and histone deacetylases during this process.

In the absence of MI, activation of p300 alone in female mice does not lead to increase in heart size or increased mortality rates at the age of 17 weeks or younger. However, LV remodeling after MI was more exaggerated in intact p300-Tg mice than in HATmut p300-Tg mice. In close association with LV remodeling, acetylation and DNA binding of GATA-4 in the MI group were higher in intact p300-Tg mice than in HATmut p300-Tg mice. The LV level of ET-1, a downstream target of GATA-4, revealed a similar tendency to its DNA binding. These findings demonstrate that a sufficient level of p300 HAT activity is critical for the acetylation and DNA binding of GATA-4 and for promotion of LV remodeling after MI in adult mice in vivo. Interestingly, despite the overexpression of p300, interstitial cell proliferation and fibrosis

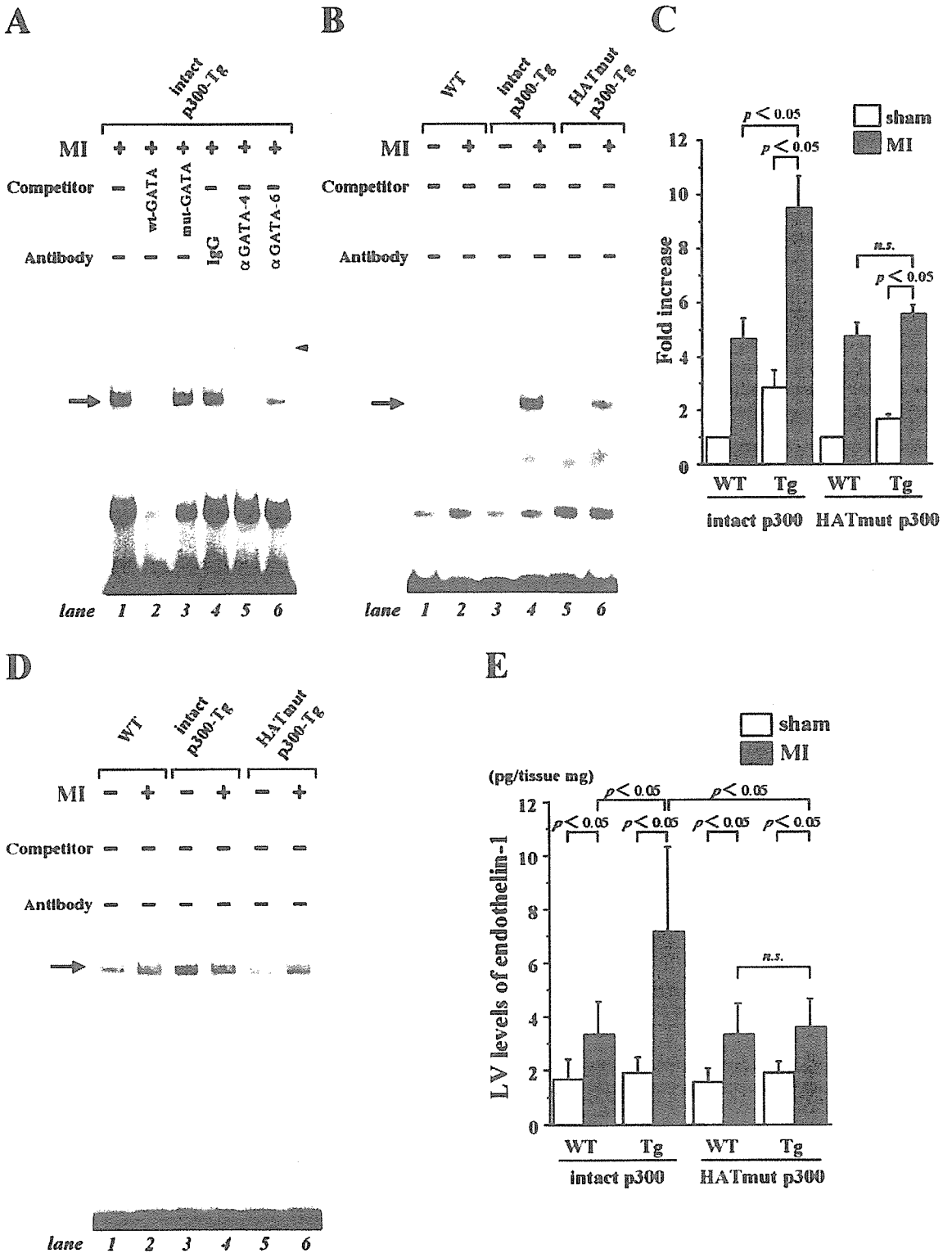


Figure 8. GATA-4/DNA binding and endothelin-1 (ET-1) level after MI in Tg and WT mouse hearts. A, Five weeks after sham or MI operation, 10 μg of protein extracts from mouse hearts were probed with a radiolabeled double-stranded oligonucleotide containing the GATA site in the ET-1 promoter. Unlabeled competitor DNAs were present at a 100-fold molar excess where indicated: lane 2, a wild-type GATA oligonucleotide (*WT-GATA*); lane 3, a mutant GATA oligonucleotide (*Mut-GATA*). Supershift assays were performed in the presence of 4 μg of control IgG, anti–GATA-4 antibody, or anti–GATA-6 antibody, as indicated (lanes 4 to 6). B and C, The amount of GATA-4/DNA binding (indicated by an arrow) was compared among extracts from each group. Representative photograph (B) and quantitative data (C) are shown. The relative amount of DNA binding in the hearts of sham-operated WT mice was set at 1.0. Values are mean±SD of 6 animals in each group. D, The same extracts used for (B) and (C) were also probed with radiolabeled double-stranded oligonucleotide containing the Sp-1 site. E, Results of LV ET-1 levels from more than 10 animals in each group are expressed as mean±SD.

remain unaffected, whereas interstitial cell proliferation is an important feature of LV remodeling. However, individual myocyte growth was remarkable in intact p300-Tg mouse hearts compared with WT mouse hearts. This observation suggests that p300 augments post-MI remodeling by affecting growth of each myocyte.

Recent studies suggest that GATA-6 as well as GATA-4 is involved in the hypertrophic response in cardiac myocytes.[26] By supershift experiments, the present study demonstrated that GATA-6 is not a major GATA factor contained in cardiac nuclear complex formed with ET-1 GATA site (Figure 8A). However, these data do not rule out the possible redundant and compensative roles of GATA-6 during LV remodeling after MI. In addition, p300 is able to interact not only with GATA-4[15,20] but also with other hypertrophy-responsive transcription factors such as myocyte enhancing factor-2,[27] nuclear factor of activated T-lymphocyte,[28] and AP-1.[29] Our findings might be applicable to these factors as well. The present study suggests that HAT activity of p300 could be a pharmacological target for LV remodeling after MI in humans. Recently, a natural compound, anacardic acid, was shown to inhibit HAT activity.[30] Therefore, it would be interesting to test whether p300 HAT inhibitors can block MI-induced LV remodeling in vivo.

Acknowledgments

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Disclosures

None.

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CLINICAL PERSPECTIVE

Left ventricular remodeling after MI consists of dynamic and biological changes of the heart. During LV remodeling, hypertrophy of each surviving myocyte occurs in proportion to infarct size and represents a major process that leads to heart failure. Hypertrophic stimuli initiate a number of subcellular signaling pathways, which finally reach the nuclei of cardiac myocytes and change the pattern of gene expression. To establish an efficient pharmacological therapy for LV remodeling, it is critical to identify a common nuclear target of hypertrophic stimuli in cardiac myocytes. One of the intrinsic histone acetyltransferases, p300, serves as a coactivator of hypertrophy-responsive transcriptional factors such as a cardiac zinc finger protein GATA-4 and is involved in its hypertrophic stimulus-induced acetylation and DNA binding. The present study demonstrates that cardiac p300 augments post-MI remodeling by affecting growth of each myocyte in adult mice in vivo. In addition, a sufficient level of p300 HAT activity was required for the acetylation and DNA binding of GATA-4 and for the promotion of LV remodeling after MI. These data suggest that HAT activity of p300 could be a pharmacological target for LV remodeling after MI in humans.

Circ J 2006; **70:** 358–361

Acute Pleuropericarditis After Coronary Stenting

— A Case Report —

Takeshi Setoyama, MD; Yutaka Furukawa, MD; Mitsuru Abe, MD;
Yoshihisa Nakagawa, MD; Toru Kita, MD; Takeshi Kimura, MD

A case of acute pleuropericarditis, which occurred after apparently successful percutaneous coronary intervention (PCI) for chronic total occlusion of the right coronary artery, is reported. The patient underwent coronary stenting without any immediate signs of complications. However, he had an acute onset of chest pain with fever which happened 4 h after PCI. He was diagnosed with acute pleuropericarditis by blood tests, electrocardiogram, chest X-ray, and echocardiogram. He rapidly recovered by intravenous hydrocortisone followed by oral prednisone administrations and percutaneous catheter pericardial drainage. Acute pleuropericarditis relevant to post-cardiac injury syndrome with an atypically early onset might have occurred in this case as a rare complication of PCI. (*Circ J* 2006; **70:** 358–361)

Key Words: Percutaneous coronary intervention; Post-cardiac injury syndrome; Prednisone; Stent

Pleuropericarditis has a variety of causes, which include viral or bacterial infections, autoimmunity, malignant neoplasm, and other cardiac insults, such as myocardial infarction and surgery or trauma to the heart. The syndrome of pleuropericarditis with fever, pleuritic chest pain, and elevated inflammatory markers secondary to cardiac injury is referred to as post-cardiac injury syndrome (PCIS). PCIS is an inflammatory process involving the pleura and pericardium and includes 2 distinct entities: post-myocardial infarction syndrome secondary to myocardial infarction and post-cardiotomy syndrome secondary to cardiac surgery or trauma.[1] PCIS after open-heart surgery is rather common and the incidence is reported as 10–50%.[2] Recently, a few reports have described PCIS occurring as a rare complication after endovascular procedures such as percutaneous coronary intervention (PCI) and trans-venous temporary/permanent pacing.[3–7] We present a case of acute pleuropericarditis which occurred after successful coronary stenting with atypically early onset for PCIS.

Case Report

A 72-year-old male hypertensive patient was admitted to hospital because of stable effort angina pectoris which appeared 2 months before admission. Treadmill stress test reproduced his chest symptoms, accompanied with reversible significant ST-segment depression in an electrocardiogram (ECG). Echocardiogram showed concentric left ventricular hypertrophy and mild left ventricular asynergy in the inferior wall. Coronary angiography revealed total occlusion in the middle right coronary artery (RCA) and 75% stenosis in the proximal RCA (Fig 1), and the distal RCA was filled through collateral circulation from the left coronary artery. The patient subsequently underwent PCI for the RCA. A 0.014 inch Miracle 3 g guidewire (Getz Bros) and a 0.014 inch PT Graphix guidewire (Boston Scientific) were used to cross the total occlusion lesion, with the aid of a microcatheter (Excelsior; Boston Scientific). The guidewire was replaced by a more flexible 0.014 inch BMW guidewire (Guidant Corporation) through the Excelsior. The lesion was predilated using a 2.0×15 mm Victo-X balloon catheter (Nippon Sherwood). Because the intravascular ultrasound examination indicated a large volume of plaque causing moderate stenoses in almost the full length of the proximal to middle RCA, 3 Cypher stents (2.5×28 mm, 3.0×33 mm, 3.5×23 mm; Johnson & Johnson) were used from the distal to the ostium of the RCA. Adjunctive postdilatation was performed with 3.0 mm and 3.5 mm stent balloons. Thus, the 75% stenotic and total occlusion lesions were successfully dilated to 0%. There were not any immediate clinical or angiographic signs of complications throughout the procedure.

Four hours after PCI, the patient complained of a mild chest pain which was augmented by deep breathing. However, his blood pressure, pulse pressure, and arterial oxygen saturation were maintained stable and ECG indicated no significant changes from his reference ECG before PCI. Thus, he was given non-steroidal anti-inflammatory drugs and put under careful course observations. The next morning (approximately 16 h after PCI), pleuritic chest pain increased with continuous fever and the patient was then diagnosed with acute pericarditis by widespread ST-segment elevation in the ECG (Fig 2) and moderate pericardial effusion in the echocardiogram (Fig 3). The chest X-ray showed cardiomegaly and blunted left costophrenic angle suggesting pleural effusion as a result of acute pleuritis (Fig 4). Blood tests indicated severe inflammation; increases in white blood cell count (21.1×10^9/L), serum C-reactive protein (CRP) level (32.1 mg/dl) and erythrocyte sedimentation rate (ESR). The serum creatine kinase level remained within a normal range. He was given 500 mg/day hydrocortisone intraveneously for 3 days and then 10 mg/day of

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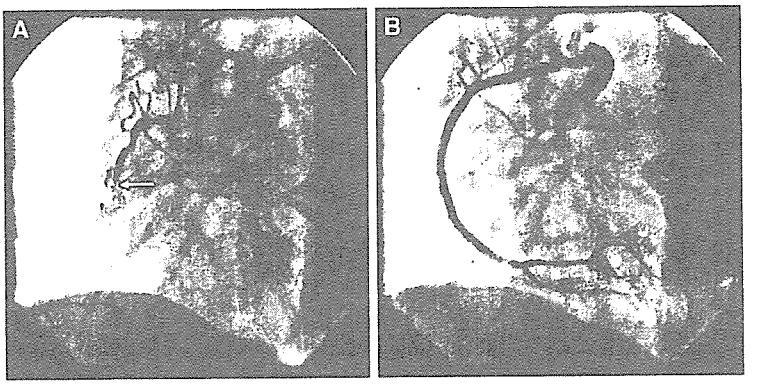

Fig 1. Percutaneous coronary intervention for the right coronary artery (RCA). (A) The middle RCA is occluded (white arrow). (B) after successful Cypher stents deployment, there is no residual stenosis in the proximal to middle RCA.

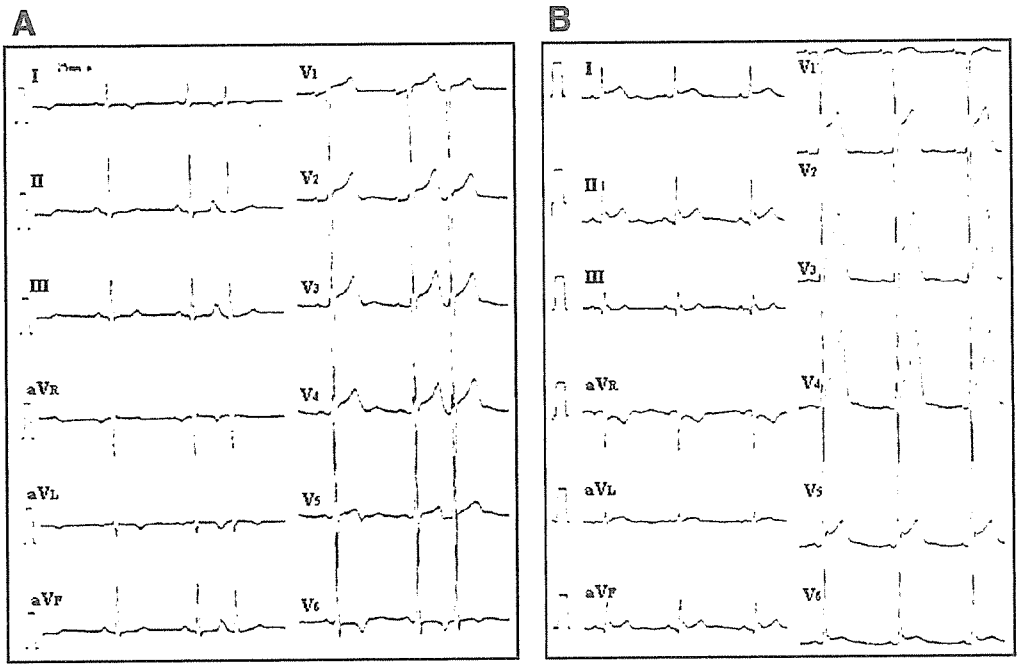

Fig 2. Electrocardiogram (ECG). (A) ECG before percutaneous coronary intervention (PCI) as a reference. (B) ECG on day 2 after PCI. Remarkable and extensive ST-segment elevations are noted.

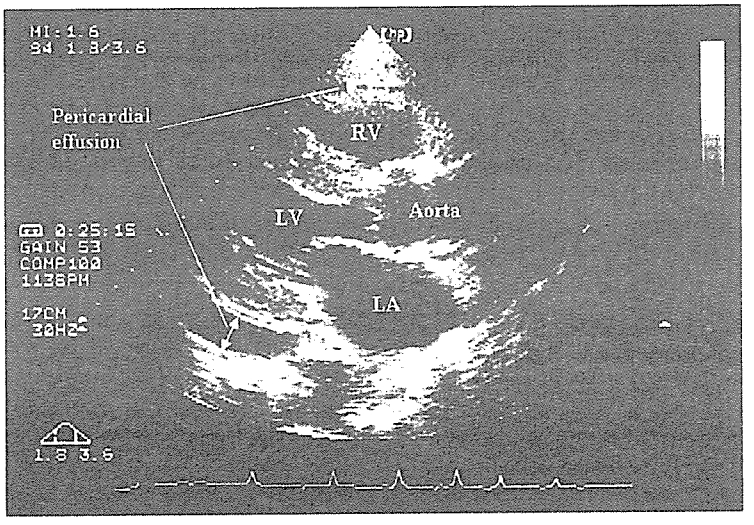

Fig 3. Echocardiogram indicating moderate pericardial effusion. RV, right ventricle; LV, left ventricle; LA, left atrium.

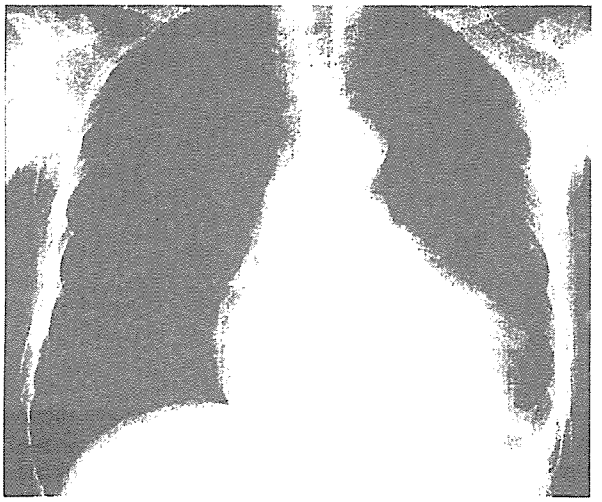

Fig 4. Chest X-ray showing cardiomegaly and the blunted left costophrenic angle. These findings coincide with electrocardiogram, echocardiogram, and clinical symptoms on the diagnosis of pleuropericarditis.

oral prednisone. He had already been given prophylactic cefazolin sodium (1 g×2/day) from the day of PCI, which was changed to piperacillin sodium (2 g×2/day) on the next day. Repeated blood cultures were both negative (sterile). When corticosteroid started, the patient's general condition was dramatically improved and chest pain was resolved, accompanied with rapid improvement of inflammatory markers and ST-segment levels in ECG. Despite the initial rapid improvement of inflammation by blood tests, the amount of pericardial effusion was still increasing, and CRP level re-elevated from 1.3 to 4.2 mg/dl, when the dose of prednisone was tapered to 5 mg/day. Therefore, prednisone was increased again to 10 mg/day, and for local control of pericardial effusion and differential diagnosis of the cause of pleuropericarditis, percutaneous catheter pericardial drainage was performed on day 21. A total of 650 ml of translucent brown-colored sterile fluid was drained. Cytology indicated that the nucleated cells were mostly lymphocytes with few neutrophils or monocytes, and no malignant cells. There were also some red blood cells and the hematocrit was approximately 3%. Circulating anti-heart antibody (AHA) level was under the detectable range, when the serum CRP level was re-elevated.

After the dose-up of corticosteroids and pericardial drainage, CRP level and ESR were finally normalized in 7 days. Prednisone was then tapered without re-elevation of inflammatory markers and the patient was discharged.

Three months after discharge, he was no longer taking corticosteroids and the recurrence of pericardial or pleural effusion was not detected by echocardiogram and chest X-ray.

Discussion

Acute pericarditis has been reported as an uncommon complication of PCI. The causes include PCIS and a stent bacterial infection because of Staphylococcus aureus or Pseudomonas aeruginosa[3-5,8-10]

The diagnosis of PCIS depends on the characteristic clinical features. The principal features of PCIS are prior injury of the pericardium and/or myocardium, latent period between the injury and the development of pericarditis or pleuropericarditis, fever, leukocytosis, elevated ESR and serum CRP level, and remarkable steroid responsiveness. PCIS includes post-myocardial infarction syndrome and post-cardiotomy syndrome. Because post-cardiotomy syndrome after cardiac surgery is relatively common, the epidemiology and pathogenesis of PCIS have been investigated in patients undergoing cardiac surgery. Three decades ago, Lessof proposed immunologic mechanisms in the pathogenesis of post-pericardiotomy syndrome[11] According to his hypothesis, release of cardiac antigens into the circulation occurs secondary to myocardial injury from infarction, surgery or trauma. This process might be facilitated by viral infection or by the presence of blood in the pericardial space. The sequelae depend on the circulating antigen levels. High circulating levels of antigens can induce autoantibodies and the formed immune complexes can deposit in the pleura, lungs, pericardium, and joints, provoking inflammation similar to other immune complex diseases[11] The remarkable responsiveness to steroids, a clinical characteristic of PCIS, indicates a possible immune-mediated mechanism in PCIS pathogenesis. In addition, the observation that the degree of pericardial/myocardial damage does not correlate with the development of PCIS also suggests an existence of another additional mechanism, ie, immune-mediated mechanism, than direct injury by infarction or trauma.

In the analysis of 19 patients undergoing cardiac surgery, anti-myosin antibodies were detected only in 7 patients who had PCIS, whereas myosin light chain and creatine kinase isoenzyme myocardial-bound were elevated to the same level in all patients[12] Similar findings were reported by De Scheerder et al in 62 patients undergoing coronary artery bypass surgery. They found a significant correlation between the frequency and intensity of the syndrome and the ratio of post-operative to pre-operative titers of anti-actin and anti-myosin antibodies[13] Contrary to these reports suggesting a pathogenetic importance of AHA, Hoffman et al reported that the development of AHA was epiphenomenon rather than cause of PCIS. They examined the time course of the appearance and disappearance of AHA in the circulation in 20 patients undergoing elective coronary artery bypass grafting. Although AHA was detected only in the sera of 3 patients who had PCIS, AHA was undetectable at the onset of PCIS in all the patients and appeared afterward in the sera[14] Moreover, AHA are not always detectable in PCIS patients[15] and the presence of the antibodies could just be a non-specific findings[16,17] Thus, the role of AHA in the pathogenesis of PCIS still remains unclear, although the association of these antibodies with PCIS has been repeatedly shown.

In the present case, circulating AHA was not detected. We measured circulating AHA level on day 18 when the serum CRP level was re-elevated by tapering of prednisone, because the dependence of the activity of pleuropericarditis on the dose of prednisone suggested possible immune-mediated mechanisms. The timing of blood sampling seemed appropriate, because Hoffman et al reported that AHA was negative at the onset of typical clinical manifestations of PCIS, became positive within 14 days from the onset, and disappeared by 90 days[14] Nevertheless, as we have already discussed, the absence of AHA does not exclude the possibility of PCIS[15] It is also possible that prednisone accelerated the disappearance of AHA, which once appeared in the serum shortly after the onset of PCIS in this case.

The clinical features of the present case were mostly consistent with the diagnostic criteria of PCIS. Prior injury of the pericardium and/or myocardium, fever, leukocytosis, elevated inflammatory markers, and remarkable steroid responsiveness were all detected in this case. In addition, PCIS could also explain a complication of pleuritis with pericarditis, because multiple serositis is commonly seen in PCIS. The only incompatible feature with the diagnosis of PCIS is the lack of latent period between the injury and the development of pleuropericarditis. Pre-immunization by recent mild myocardial injury might explain for this incompatibility, because the patient's angina appeared only a few months before the event of pleuropericarditis after PCI and the occlusion of the RCA was possibly responsible for the occurrence of his angina pectoris. The color of the drained pericardial effusion was translucent brown and contained some red blood cells, suggesting that a small amount of bleeding into pericardial space had occurred. Because the presence of blood in the pericardial space facilitates the development of PCIS, mild injuries of the coronary artery during PCI, such as unrecognized perforation by a guidewire, might cause small bleeding into the pericardial space and trigger PCIS in the pre-immunized patient.

Another possible cause is purulent pericarditis because of stent infection. The most common pathogen of purulent pericarditis associated with PCI is Staphylococcus aureus, but Pseudomonas aeruginosa can also cause stent infection[9,10] In the present case, neither of these pathogens appeared to cause pleuropericarditis, because prophylactic cefazolin sodium could have possibly prevented staphylococcal infection. Corticosteroids, rather than piperacillin sodium, was effective for the improvement of symptom and laboratory data of inflammation. Dramatic effects of corticosteroids and re-elevation of serum CRP level when prednisone was tapered, strongly suggest the immune-mediated mechanisms in the pathogenesis of pleuropericarditis in this case. Such a remarkable response to corticosteroid is one of typical clinical features of PCIS rather than purulent pericarditis. Finally, repeated blood cultures and a culture of pericardial effusion were all negative (sterile), which disagrees with purulent pericarditis.

In summary, acute pleuropericarditis occurred after apparently successful PCI for the total occlusion lesion. The clinical features were suitable for the diagnosis of PCIS except the atypically early onset. The patient was fully recovered by the treatment with corticosteroids.

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Gender Difference in Coronary Events in Relation to Risk Factors in Japanese Hypercholesterolemic Patients Treated With Low-Dose Simvastatin

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Background Gender differences between the risk factors for coronary heart disease and coronary events were examined in the Japan Lipid Intervention Trial, a 6-year observational study.

Methods and Results Men (12,575) and women (27,013) were analyzed for risk of coronary events (acute myocardial infarction and sudden cardiac death). Simvastatin reduced serum low-density lipoprotein cholesterol (LDL-C) by 27% in both genders, and increased serum high-density lipoprotein cholesterol (HDL-C) in men (5%) and women (4%). The incidence of coronary events was lower in women (0.64/1,000 patient-years) than in men (1.57/1,000 patient-years). The risk of coronary events increased by 18% in men and 21% in women with each 10 mg/dl elevation of LDL-C, and decreased by 39% in men and 33% in women with each 10 mg/dl elevation of HDL-C. The risk increased proportionally with aging in women, but not in men. Diabetes mellitus (DM) was more strongly related to the risk of coronary events for women (relative risk 3.07) than for men (relative risk 1.58).

Conclusions The incidence of coronary events is lower in women. Serum LDL-C is related to an increased risk of coronary events to the same extent in both genders. DM seems to be a more important risk factor in women, trading off the lower risk of coronary events among them. (*Circ J* 2006; **70:** 810–814)

Key Words: Coronary events; Hyperlipidemia; Risk factors; Serum cholesterol; Sex differences

Coronary heart disease (CHD), including myocardial infarction and cardiac sudden death, is one of the leading causes of death in Japan.[1] The risk of developing CHD is known to be markedly different between men and women:[2,3] CHD incidence is 2 to 5 times higher among middle-aged men than women. In the Japan Lipid Intervention Trial (J-LIT);[4–7] we previously reported that serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations were positively and serum high-density lipoprotein cholesterol (HDL-C) concentration was inversely related to CHD or cerebrovascular disease risk in patients under treatment for hypercholesterolemia. The role of coronary risk factors in the development of CHD has been studied extensively in men;[8–10] but relatively few studies have investigated women.[2,11]

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This study aimed to assess gender differences in the association of risk factors with CHD in the J-LIT data. The J-LIT is a nationwide cohort study of 52,421 hypercholesterolemic patients treated with open-labeled low-dose simvastatin (5–10 mg/day).[4,5] The J-LIT included a large number of female patients, and we were able to investigate the gender difference in the role of risk factors in the occurrence of coronary events.

Methods

Study Design

The design of the J-LIT study has been previously described.[12] Briefly, study patients with serum TC concentration ≥220 mg/dl, men aged 35–70 years and postmenopausal women aged 70 years or less, were treated with 5–10 mg/day of simvastatin. Body weight, serum lipid concentrations (TC, LDL-C, HDL-C, and triglyceride (TG)) were measured at baseline, and patients were interviewed as regards family history of CHD, number of cigarettes smoked, and the amount of alcohol ingestion. Serum lipid concentrations and CHD-related events (acute myocardial infarction and cardiac sudden death) were monitored every 6 months for 6 years in all patients, including those who discontinued simvastatin. Serum lipid concentrations were determined in each study institution, and the serum LDL-C concentration was calculated using the Friedewald formula for patients with TG concentration ≤400 mg/dl.[13] Study physicians recommended dietary and exercise-therapy for hyperlipidemia to all patients. Additional lipid-lowering

agents were allowed only when an adequate response in serum TC concentration was not gained by simvastatin monotherapy. Each patient was informed of the purpose and method of the study, drug efficacy and the need for long-term treatment and they gave verbal, not written, informed consent.

Subjects

Patients who had been previously treated with a lipid-lowering agent were screened for eligibility after a washout period of at least 4 weeks. For patients previously treated with probucol, the washout period was at least 12 weeks. The exclusion criteria were the occurrence of acute myocardial infarction or stroke within the past month, concurrent uncontrolled diabetes mellitus (DM), serious hepatic or renal disease, secondary hypercholesterolemia, cancer or any other illness with potentially poor survival.

Of the 52,421 patients enrolled, 5,127 were excluded because of a history of CHD, 4,934 for lack of follow-up data, and 2,772 for missing data of the covariates. Therefore, data from 39,588 patients (12,575 men, 27,013 women) were used in the present study.

Endpoints

The primary endpoints were major coronary events, defined as nonfatal and fatal myocardial infarction and sudden cardiac death. Incidence of myocardial infarction or death was counted once for each patient during the treatment, and the follow-up data thereafter were excluded from the analysis. The events were reviewed and determined by the Endpoint Classification Committee.

Statistical Analysis

The mean lipid concentrations were calculated using data available at the follow-up points in time during the treatment period. The data of lipid concentrations after the onset of events were excluded. Data during the treatment period after discontinuation of simvastatin were also included for analysis. Mean values for serum lipid concentrations and age were tested with unpaired t-test, and the prevalence of baseline characteristics were tested with the chi-square test for comparison between men and women. Patients in each sex were categorized into 5–6 groups according to the mean lipid concentrations of treatment period for TC, TG, LDL-C and HDL-C with intervals of 20, 50, 20, 10 mg/dl, respectively, and for the LDL-C/HDL-C ratio with an interval of 0.5. The reference category for the relative risk was set on the group with the lowest lipid concentrations and the lowest value of LDL-C/HDL-C ratio. Relative risks and the 95% confidence intervals (CI) were calculated using the Cox proportional hazards model with adjustment for baseline characteristics such as sex, age, hypertension, DM, body mass index (BMI), ECG abnormality, family history of CHD, alcohol ingestion and cigarette smoking. Heterogeneity between men and women was evaluated by the likelihood ratio test. Two-sided p-value <0.05 was considered statistically significant. All the statistical calculations were performed using SAS software (version 8.02, SAS Institute, Inc, Cary, NC, USA).

Results

Serum Lipids and Other Risk Factors

There were no significant difference as regards the prevalence of obesity (BMI ≥25.0 kg/m^2), hypertension, ECG abnormality, and family history of CHD between men and women (Table 1). In men, the prevalence of DM was higher (p<0.001), and cigarette smoking and alcohol ingestion were much more frequent (p<0.001).

Table 1 Baseline Characteristics of the Subjects

	Men (n=12,575)	*Women (n=27,013)*
Age (years)	*54.0 (9.1)*	*59.5 (6.5)*
Obesity (%)[a]	*36.7*	*32.2*
Hypertension (%)[b]	*45.4*	*46.3*
Diabetes mellitus (%)[c]	*20.0*	*13.9*
ECG abnormality (%)[d]	*13.4*	*12.9*
Family history of CHD (%)[e]	*5.1*	*4.8*
Cigarette smoking (%)[e]	*43.8*	*4.1*
Alcohol use (%)[e]	*73.4*	*8.7*
Lipid profiles		
Baseline (mg/dl)		
TC	*268 (41)*	*271 (31)*
LDL-C	*178 (34)*	*184 (33)*
TG	*250 (241)*	*169 (111)*
HDL-C	*49 (15)*	*55 (15)*
During the treatment (mg/dl)		
TC	*218 (31)*	*221 (29)*
LDL-C	*130 (31)*	*135 (28)*
TG	*198 (133)*	*148 (77)*
HDL-C	*51 (13)*	*57 (14)*

Figs are mean±SD unless otherwise specified.
CHD, coronary heart disease; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol.
[a] Body mass index ≥25 kg/m^2. [b] Systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥95 mmHg or medication for hypertension. [c] Fasting plasma glucose ≥140 mg/dl or medication. [d] Study physician's diagnosis. [e] Self-reported information.

Lipid profiles at baseline and during the treatment period are shown for men and women in Table 1. Men had higher concentrations of serum TG and lower concentrations of serum HDL-C at baseline and during the treatment in comparison with women. Mean percent changes in the TC, LDL-C, TG, and HDL-C concentrations from baseline to during the treatment in men were −18.8% (p<0.001), −27.2% (p<0.001), −20.9% (p<0.001), and +4.7% (p<0.001), respectively, and the corresponding values in women were −18.2% (p<0.001), −26.6% (p<0.001), −12.8% (p<0.001) and +4.4% (p<0.001), respectively.

Incidence of Coronary Events

The incidence of coronary events was greater (105/12,575) in men than in women (93/27,013) during the treatment period. Incidence rates of coronary events per 1,000 patient-years were 1.57 in men and 0.64 in women. The age-adjusted relative risk of coronary events for men vs women was 2.81 (95% CI 2.10–3.76, p<0.001).

Serum Lipid Concentrations During the Treatment Period and Risk of Coronary Events

The risk of coronary events in relation to serum lipid concentrations is shown in Table 2. Increased risk for coronary events was evident at TC ≥240 mg/dl and LDL-C ≥160 mg/dl in both men and women. An increased risk of CHD associated with elevated concentration of TG (≥250 mg/dl) was noted in women but not in men. In men, the relationship between TG and CHD risk was not measurable. A lower risk of coronary events associated with elevation in HDL-C was seen in both sexes, but the protec-

Table 2 Relative Risk of Coronary Events According to Serum Lipid Concentrations During Treatment[a]

	Men					Women				
	N	*Event*	*RR*	*95%CI*	*p value*	*N*	*Event*	*RR*	*95%CI*	*p value*
TC (mg/dl)										
<200	*3,442*	*24*	*1.00*	*(Referent)*		*5,833*	*22*	*1.00*	*(Referent)*	
200–219	*3,643*	*23*	*0.99*	*(0.56–1.77)*	*0.984*	*8,194*	*14*	*0.52*	*(0.27–1.02)*	*0.057*
220–239	*3,029*	*25*	*1.46*	*(0.83–2.56)*	*0.192*	*7,070*	*18*	*0.88*	*(0.47–1.64)*	*0.687*
240–259	*1,431*	*15*	*2.01*	*(1.05–3.88)*	*0.036*	*3,668*	*22*	*2.19*	*(1.21–3.98)*	*0.010*
260–	*1,030*	*18*	*3.48*	*(1.86–6.52)*	*<0.001*	*2,248*	*17*	*2.82*	*(1.48–5.36)*	*0.002*
LDL-C (mg/dl)										
<120	*4,680*	*27*	*1.00*	*(Referent)*		*8,050*	*22*	*1.00*	*(Referent)*	
120–139	*3,542*	*23*	*1.24*	*(0.71–2.16)*	*0.456*	*8,418*	*17*	*0.83*	*(0.44–1.57)*	*0.566*
140–159	*2,406*	*21*	*1.84*	*(1.03–3.26)*	*0.038*	*6,185*	*19*	*1.42*	*(0.77–2.64)*	*0.263*
160–179	*1,057*	*12*	*2.60*	*(1.31–5.17)*	*0.006*	*2,673*	*17*	*3.29*	*(1.74–6.23)*	*<0.001*
180–	*648*	*17*	*6.58*	*(3.53–12.25)*	*<0.001*	*1,564*	*17*	*5.78*	*(3.03–11.00)*	*<0.001*
TG (mg/dl)										
<100	*1,521*	*11*	*1.00*	*(Referent)*		*6,337*	*18*	*1.00*	*(Referent)*	
100–149	*3,663*	*22*	*0.84*	*(0.41–1.74)*	*0.634*	*10,444*	*32*	*0.98*	*(0.55–1.76)*	*0.946*
150–199	*3,127*	*33*	*1.51*	*(0.76–3.02)*	*0.243*	*5,861*	*17*	*0.87*	*(0.44–1.71)*	*0.684*
200–249	*1,768*	*18*	*1.46*	*(0.68–3.15)*	*0.330*	*2,429*	*9*	*1.12*	*(0.50–2.53)*	*0.783*
250–	*2,494*	*21*	*1.24*	*(0.58–2.65)*	*0.572*	*1,921*	*17*	*2.62*	*(1.32–5.21)*	*0.006*
HDL-C (mg/dl)										
<40	*2,198*	*36*	*1.00*	*(Referent)*		*1,758*	*10*	*1.00*	*(Referent)*	
40–44	*2,133*	*23*	*0.64*	*(0.38–1.09)*	*0.099*	*2,794*	*17*	*1.12*	*(0.51–2.45)*	*0.776*
45–49	*2,207*	*17*	*0.44*	*(0.25–0.80)*	*0.006*	*4,101*	*24*	*1.09*	*(0.52–2.28)*	*0.819*
50–54	*1,956*	*13*	*0.39*	*(0.21–0.74)*	*0.004*	*4,440*	*13*	*0.57*	*(0.25–1.30)*	*0.179*
55–59	*1,402*	*8*	*0.33*	*(0.15–0.72)*	*0.005*	*4,053*	*13*	*0.66*	*(0.29–1.51)*	*0.324*
60–	*2,679*	*8*	*0.17*	*(0.08–0.36)*	*<0.001*	*9,867*	*16*	*0.33*	*(0.15–0.73)*	*0.006*
LDL-C/HDL-C										
<2.0	*2,851*	*11*	*1.00*	*(Referent)*		*7,426*	*11*	*1.00*	*(Referent)*	
2.0–2.4	*2,719*	*11*	*1.10*	*(0.48–2.55)*	*0.817*	*6,909*	*19*	*1.95*	*(0.92–4.10)*	*0.080*
2.5–2.9	*2,598*	*17*	*1.91*	*(0.89–4.10)*	*0.095*	*5,884*	*14*	*1.68*	*(0.76–3.72)*	*0.199*
3.0–3.4	*1,889*	*20*	*3.21*	*(1.53–6.74)*	*0.002*	*3,545*	*21*	*4.57*	*(2.19–9.54)*	*<0.001*
3.5–4.0	*1,082*	*13*	*3.87*	*(1.72–8.72)*	*0.001*	*1,728*	*12*	*5.04*	*(2.21–11.49)*	*<0.001*
4.0–	*1,194*	*28*	*8.06*	*(3.95–16.44)*	*<0.001*	*1,398*	*15*	*8.56*	*(3.88–18.88)*	*<0.001*

RR, relative risk; CI, confidence interval. Other abbreviations see in Table 1.

[a] Coronary events included acute myocardial infarction and sudden cardiac death. Adjustment for age, hypertension, diabetes mellitus, body mass index, ECG abnormality, family history of CHD, cigarette smoking, and alcohol use.

Table 3 Relative Risk of Coronary Events and Baseline Characteristics[a]

	Men					Women					*Heterogeneity p value[b]*
	N	*Event*	*RR*	*95%CI*	*p value*	*N*	*Event*	*RR*	*95%CI*	*p value*	
Age (years)											
<55	*6,281*	*49*	*1.00*	*(Referent)*		*6,137*	*8*	*1.00*	*(Referent)*		*0.008*
55–59	*2,182*	*14*	*0.74*	*(0.41–1.34)*	*0.320*	*6,488*	*15*	*1.82*	*(0.77–4.29)*	*0.174*	
60–64	*2,164*	*17*	*0.87*	*(0.50–1.53)*	*0.627*	*7,112*	*29*	*3.02*	*(1.38–6.62)*	*0.006*	
≥65	*1,948*	*25*	*1.42*	*(0.86–2.34)*	*0.168*	*7,276*	*41*	*4.11*	*(1.92–8.82)*	*<0.001*	
Obesity[c]	*4,621*	*40*	*0.99*	*(0.66–1.48)*	*0.956*	*8,700*	*32*	*0.91*	*(0.59–1.40)*	*0.663*	*0.676*
Hypertension[d]	*5,705*	*68*	*2.15*	*(1.42–3.26)*	*<0.001*	*12,511*	*62*	*2.05*	*(1.32–3.18)*	*0.001*	*0.864*
Diabetes mellitus[e]	*2,513*	*29*	*1.58*	*(1.03–2.43)*	*0.037*	*3,747*	*31*	*3.07*	*(1.99–4.74)*	*<0.001*	*0.019*
ECG abnormality[f]	*1,681*	*26*	*1.86*	*(1.18–2.91)*	*0.007*	*3,473*	*23*	*1.67*	*(1.04–2.70)*	*0.035*	*0.972*
Family history of CHD[g]	*637*	*10*	*2.00*	*(1.04–3.84)*	*0.038*	*1,289*	*13*	*3.34*	*(1.85–6.04)*	*<0.001*	*0.317*
Cigarette smoking[g]	*5,506*	*52*	*1.46*	*(0.98–2.17)*	*0.063*	*1,105*	*9*	*2.94*	*(1.43–6.02)*	*0.003*	*0.148*
Alcohol use[g]	*9,224*	*70*	*0.63*	*(0.41–0.96)*	*0.031*	*2,337*	*6*	*0.61*	*(0.26–1.45)*	*0.266*	*0.933*

Abbreviations see in Tables 1,2.

[a] Coronary events included acute myocardial infarction and sudden cardiac death. Adjustment for age, hypertension, diabetes mellitus, body mass index, ECG abnormality, family history of CHD, cigarette smoking, and alcohol use. [b] Heterogeneity between men and women, based on the likelihood ratio test. [c] Body mass index ≥25 kg/m². [d] Systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥95 mmHg or medication for hypertension. [e] Fasting plasma glucose ≥140 mg/dl or medication. [f] Study physician's diagnosis. [g] Self-reported information.

tive association was more evident in men. The relative risk for coronary events was substantially increased in patients with LDL-C/HDL-C ≥3.0 in both men and women.

The increase in the risk of coronary events for each 10 mg/dl elevation of LDL-C concentration during the treatment period was 18% (95% CI 12–24%) in men and 21% (95% CI 15–27%) in women, and the decrease in CHD risk associated with each 10 mg/dl elevation of HDL-C concentration was 39% in men and 33% in women. The relationships of coronary events with baseline LDL-C and HDL-C concentrations were also examined, but were much weaker than those observed during the treatment period. With each 10 mg/dl elevation of LDL-C concentration at baseline, the increase in the relative risk was 7% for men

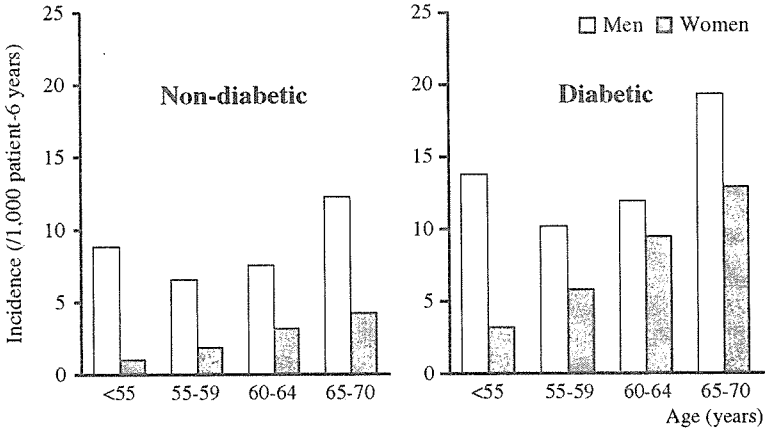

Fig 1. Estimated rates of coronary events according to age in men and women with and without diabetes mellitus (DM). Incidence rates were calculated from coronary heart disease (CHD) relative risks and the proportion of patients in each age category, for men and women separately, using Cox proportional hazards model, in which adjustment was made for age, hypertension, DM, body mass index, ECG abnormality, family history of CHD, cigarette smoking, and alcohol use.

and 9% for women and the decrease in risk with each 10 mg/dl elevation of HDL-C at baseline was 20% in both men and women.

Patient Baseline Characteristics and Risk of Coronary Events

The effect of age on the risk of coronary events was seen in women, but not in men (Table 3). Hypertension, DM, ECG abnormalities and a family history of CHD were also risk factors for coronary events in both men and women, but increased risks associated with DM and a family history of CHD were more marked for women than for men; the relative risk with DM was 1.58 in men and 3.07 in women, and the corresponding values for a family history of CHD were 2.00 in men and 3.34 in women. Obesity was unrelated to coronary events in either men or women. Although alcohol ingestion was protective in both men and women to the same extent, cigarette smoking was more strongly related to an increased risk of coronary events in women.

Discussion

This report addresses the gender differences in the relationship of serum lipid concentrations and other risk factors to CHD risk in Japanese patients under long-term treatment for hypercholesterolemia. Although serum TC and LDL-C concentrations were very similarly related to CHD risk in men and women, there was a difference between men and women in the relationship to serum TG and HDL-C concentrations. An inverse relationship of HDL-C to CHD risk was seen in men and women, but the HDL-C concentration showing a decreased risk of CHD differed by sex. The risk was significantly decreased at HDL-C ≥45 mg/dl in men and at HDL-C ≥60 mg/dl in women. The findings agree with observations published in the United States and Europe[2,3] and further indicate that the criterion of "low HDL-C" must be differential for men and women. An increased risk was observed only in women with an extremely high concentration of TG (≥250 mg/dl). Interpretation of this finding is difficult, and we do not have a clear idea about the implication of the present finding on serum TG.

In the present study, men did not show a clear increase in the risk of coronary events with increasing age, whereas there was a progressive increase in the risk with advancing age in women. The latter finding could be a reflection of the increase in serum TC and LDL-C concentrations with increasing age after menopause. The lack of an increasing trend in the association between age and coronary events in men is an unexpected finding, and may have been due to unknown characteristics of the male participants in the present study.

Whereas DM was related to increased CHD risk in both men and women, the increased risk was much greater in women, as indicated by a statistically significant interaction (p=0.019). These results did not change when further adjusted for TC or LDL-C. However, the risk difference between men and women for DM was not unique to the J-LIT patients. In a meta-analysis of 10 prospective studies, Lee et al showed that the effect of DM on the CHD risk was greater in women than in men.[14] They showed that the relative risk of coronary death for DM patients vs non-DM patients was 2.58 (95% CI 2.05–3.26) in women and 1.85 (95% CI 1.47–2.33) in men (interaction p=0.045).[14] It was further noted in a later study that DM diminished the female advantage for lower CHD incidence.[15] That DM is a stronger CHD risk factor in women may be related to the lower concentrations of HDL-C. Walden suggested that lower HDL-C concentrations in diabetic women as compared with men might be relevant to a stronger association between DM and CHD in women.[16] In the present study, mean HDL-C concentrations in female diabetic patients were lower than those of non-diabetic patients (55.5 vs 57.5 mg/dl, p<0.001), but there was no difference in the HDL-C concentrations between the 2 groups in men (50.8 vs 51.3 mg/dl, p=0.09). The relative risk for DM was unchanged with adjustment for HDL-C. When the predicted rates of CHD incidence according to age were examined in men and women with and without DM (Fig 1), the increase in CHD incidence with aging was augmented in the presence of DM. Notably, DM diminished the women's advantage of having a lower CHD incidence in older patients.

Both cigarette smoking and family history of CHD were related to a greater increase in the risk of coronary events in women than in men. These differential increases in men and women may have been caused by random variation, as indicated by the lack of statistical significance for the interaction. As regards the effect of cigarette smoking, some studies suggest that smoking is a stronger risk factor in women than in men[2,17] but others have failed to find such a finding.[18]

Finally, the present study results indicated that hypertension was an important risk factor in men and women equally, and that alcohol ingestion was protective in both sexes. These findings are in agreement with observations reported elsewhere.[19–21]

In conclusion, the incidence of coronary events was 60% lower in women than in men among the J-LIT participants. Although the relationship of serum TC and LDL-C concentrations to coronary events was similar in men and women, the HDL-C concentration associated with a decreased risk of coronary events was slightly higher in women. DM was a stronger risk factor in women, and traded off the women's advantage of having a lower risk of coronary events, especially in aged patients.

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Serofendic Acid, a Novel Substance Extracted From Fetal Calf Serum, Protects Against Oxidative Stress in Neonatal Rat Cardiac Myocytes

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OBJECTIVES We examined whether serofendic acid (SFA) has protective effects against oxidative stress in cardiac myocytes.

BACKGROUND We previously identified a novel endogenous substance, SFA, from a lipophilic extract of fetal calf serum. Serofendic acid protects cultured neurons against the cytotoxicity of glutamate, nitric oxide, and oxidative stress.

METHODS Primary cultures of neonatal rat cardiac myocytes were exposed to oxidative stress (H_2O_2, 100 μmol/l) to induce cell death. Effects of SFA were evaluated with a number of markers of cell death.

RESULTS Pretreatment with SFA (100 μmol/l) significantly suppressed markers of cell death, as assessed by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling staining and cell viability assay. Loss of mitochondrial membrane potential ($\Delta\Psi_m$) is a critical step of the death pathway, which is triggered by matrix calcium overload and reactive oxygen species. Serofendic acid prevented the $\Delta\Psi_m$ loss induced by H_2O_2 in a concentration-dependent manner (with saturation by 100 μmol/l). Serofendic acid remarkably suppressed the H_2O_2-induced matrix calcium overload and intracellular accumulation of reactive oxygen species. The protective effect of SFA was comparable to that of a mitochondrial adenosine triphosphate-sensitive potassium ($mitoK_{ATP}$) channel opener, diazoxide. Furthermore, $mitoK_{ATP}$ channel blocker, 5-hydroxydecanoate (500 μmol/l), abolished the protective effect of SFA. Co-application of SFA (100 μmol/l) and diazoxide (100 μmol/l) did not show an additive effect. Thus, SFA inhibited the oxidant-induced mitochondrial death pathway, presumably through activation of the $mitoK_{ATP}$ channel.

CONCLUSIONS Serofendic acid protects cardiac myocytes against oxidant-induced cell death by preserving the functional integrity of mitochondria. (J Am Coll Cardiol 2006;47:1882–90) © 2006 by the American College of Cardiology Foundation

Mitochondria play critical roles in cell death in response to a variety of stresses, such as myocardial ischemia/reperfusion (1–3). Opening of the mitochondrial permeability transition pore (MPTP), a non-specific pore that opens at the contact site between outer and inner mitochondrial membranes, results in the loss of mitochondrial membrane potential ($\Delta\Psi_m$), matrix swelling, and the release of cytochrome *c* and other proapoptotic factors that lead to cell death (4–6). Mitochondrial matrix calcium ($[Ca^{2+}]_m$) overload and reactive oxygen species (ROS) favor MPTP opening (7). Inhibition of MPTP opening by preventing $[Ca^{2+}]_m$ overload and ROS generation will be an effective strategy for the protection of hearts from ischemia/reperfusion injury.

We have shown that adenosine triphosphate–sensitive potassium channels located in the inner mitochondrial membrane ($mitoK_{ATP}$ channels) play a central role in the signaling cascade of protection against oxidative stress in the cardiac ventricular myocytes (8,9) and the cerebellar granule neurons (10,11). $MitoK_{ATP}$ channels prevent $[Ca^{2+}]_m$ overload and ROS generation, thereby inhibiting the MPTP opening in both types of cells (10,12–14). Diazoxide, a selective opener of $mitoK_{ATP}$ channels, has been shown to have protective effects against myocardial ischemia/reperfusion both in vitro (15,16) and in vivo (17,18). Unfortunately, the clinical use of this agent has been hampered, owing to unwanted side effects, such as excessive hypotension or edema.

We previously purified a novel neuroprotective substance named "serofendic acid" (SFA) derived from the lipophilic fraction of fetal calf serum (19). The compound exhibited the ability to protect cultured cortical and striatal neurons against glutamate, nitric oxide, and H_2O_2 cytotoxicity (19–22). Given that H_2O_2 is also responsible for the tissue injury during myocardial ischemia/reperfusion, we hypothesized that SFA might have cardioprotective effects against ischemia/reperfusion injury. In the present study, we investigated whether SFA has protective effects against oxidative stress in neonatal rat cardiac myocytes.

METHODS

All procedures were performed in accordance with the Kyoto University animal experimentation committee, which

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Abbreviations and Acronyms

5-HD	= 5-hydroxydecanoate
$\Delta\Psi_m$	= mitochondrial membrane potential
$[Ca^{2+}]_m$	= mitochondrial matrix calcium
DAPI	= 4',6-diamidino-2-phenylindole
DCF	= chloromethyl-2,7-dichlorodihydrofluorescein diacetate
DMEM	= Dulbecco's Modified Eagle Medium
FACS	= fluorescence-activated cell sorter
$MitoK_{ATP}$	= mitochondrial adenosine triphosphate-sensitive potassium
MPTP	= mitochondrial permeability transition pore
ROS	= reactive oxygen species
SFA	= serofendic acid
TMRE	= tetramethylrhodamine ethyl ester
TUNEL	= terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling

conforms to the *Guide for the Care and Use of Laboratory Animals*, published by the National Institutes of Health.

Primary culture of neonatal rat cardiac ventricular myocytes. Cardiac ventricular myocytes were prepared from one- to two-day-old Wistar rats and cultured as previously described (8). In brief, the hearts were removed, and the ventricles were minced into small fragments, which were digested by trypsin dissociation. The dissociated cells were preplated for 1 h to enrich the culture with myocytes. The non-adherent myocytes (approximately, 30 to 50 million cells per isolation) were then plated in plating medium consisting of Dulbecco's Modified Eagle Medium (DMEM) (Nacalai Tesque, Kyoto, Japan) supplemented with 5% fetal calf serum, penicillin (100 U/ml), streptomycin (100 mg/ml), and 2 μg/ml vitamin B12. The final myocyte cultures contained >90% cardiac myocytes at partial confluence. The cells were maintained at 37°C in the presence of 5% CO_2 in a humidified incubator. Bromodeoxyuridine (0.1 mmol/l) was incubated in the medium for the first three days after plating to inhibit fibroblast growth. Cultures were then placed in serum-free DMEM containing vitamin B12 and transferrin 24 h before the drug treatment.

Experimental protocol. Neonatal rat cardiac myocytes in primary culture were randomly assigned to one of three experimental groups: 1) control group; 2) incubation with 100 μmol/l H_2O_2 for 60 min; and 3) pretreated 100 μmol/l SFA for 30 min, followed by 100 μmol/l H_2O_2 for 60 min. At the beginning of the experiment, culture media were replaced with fresh serum-free DMEM containing those drugs, and the cells were exposed to those drugs during the entire experimental period.

The SFA was dissolved in dimethyl sulfoxide to make 100 mmol/l stock solution before being added into experimental solution. The final concentration of dimethyl sulfoxide was <0.1%.

MTS assay. Cell viability was quantified on the basis of metabolic activity with the MTS assay (Promega, Madison, Wisconsin), according to manufacturer's protocol. The cultures were incubated in serum-free medium containing 20 μl/well of the MTS tetrazolium compound for 3 h at 37°C. The absorbance of formazan products was photometrically measured at 490 nm with a microplate reader, ARVOsx (PerkinElmer, Shelton, Washington). The cell viability was expressed as the percentage of the absorbance measured in the control group.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining. The TUNEL staining was performed according to the manufacturer's protocol (Roche, Indianapolis, Indiana). Fluorescein labels incorporated in nucleotide polymers were detected with a fluorescence microscope (Axioskop 2 plus; Zeiss, Thornwood, New York).

4',6-diamidino-2-phenylindole (DAPI) staining. Cells were stained with the deoxyribonucleic acid binding dye DAPI (5 μmol/l; Molecular Probes, Eugene, Oregon). The nuclear morphology of cells were visualized and photographed with the fluorescence microscope.

Loading of cells with fluorescent indicator. To monitor $\Delta\Psi_m$, cells were loaded with tetramethylrhodamine ethyl ester (TMRE) (Molecular Probes) 100 nmol/l at 37°C for 20 min. To monitor $[Ca^{2+}]_m$, the cells were loaded with 2 μmol/l rhod-2 AM (Molecular Probes) at 37°C for 30 min. We assayed the intracellular ROS production with chloromethyl-2,7-dichlorodihydrofluorescein diacetate (DCF) (Molecular Probes). Cells were loaded with 4 μmol/l DCF at 37°C for 30 min, and the formation of the oxidized derivative was monitored by the increase of green fluorescence.

Fluorescence-activated cell sorter (FACS) analysis. Cells plated on regular six-well plates (1.0 to 1.5 million cells per well) were used for the FACS analysis of $\Delta\Psi_m$. The TMRE-loaded cells were harvested by tripsinization at the end of the experimental protocols and analyzed with FACSAria (BD Biosciences, San Jose, California) (20,000 cells/sample). The fluorescence intensity of TMRE was monitored at 582 nm (FL-2). The FACS data were analyzed with analysis software (WinMDI).

Confocal imaging. Cells plated on 35-mm glass-bottom dishes (1.0 to 1.5 million cells per dish) were maintained at 37°C in the presence of 5% CO_2 with a heater platform installed on a microscope stage and were placed in serum-free DMEM. After the desired temperature was reached, time-lapse confocal microscopy was started with 2-min intervals, with a 20× objective lens. Images were taken with laser scanning confocal microscopy (LSM510, Zeiss). The TMRE and rhod-2 AM was excited with a 543 nm line of a helium/neon laser. The DCF was excited with a 488 nm line of an argon laser.

Twenty-five cells were randomly selected in each scan by drawing regions around individual cells, and the red or green fluorescence intensity was sequentially monitored.

Image analysis. Quantitative image analysis was performed with an image analysis software (ImageJ).

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Statistical analysis. All the quantitative data are presented as the mean ± SEM. Multiple comparisons among groups were carried out by one-way analysis of variance with Bonferroni's post-hoc test. A level of $p < 0.05$ was accepted as statistically significant.

RESULTS

Figure 1A demonstrates TUNEL staining and DAPI staining in each experimental group. The TUNEL staining detects nuclear deoxyribonucleic acid strand breaks, which occur during the terminal phase of apoptosis. Control cells exhibited few TUNEL-positive nuclei, but exposure to 100 μmol/l H_2O_2 for 16 h increased the number of TUNEL-positive nuclei, which can be seen as bright spots, indicating enhanced apoptosis under the treatment with H_2O_2. There were obviously fewer TUNEL-positive nuclei in the SFA-treated group, despite the similar density of cells compared with the H_2O_2 group. Cells incubated with 100 μmol/l H_2O_2 for 16 h were also stained with deoxyribonucleic acid binding dye DAPI. Fragmented or shrunken nuclei were observed in the H_2O_2 group, but SFA displayed a significant protective effect in the preservation of nuclear morphology. Figure 1B shows a quantitative determination of TUNEL-positive nuclei in each experimental group. The data indicate that SFA has a significant protective effect against H_2O_2-induced nuclear damage.

We examined whether SFA would affect the overall viability of cultured cardiac myocytes exposed to oxidative stress. The MTS assay revealed that 100 μmol/l SFA partly but significantly protected against H_2O_2-induced cytotoxicity (Fig. 1C).

Loss of $\Delta\Psi_m$ is a critical event early in the process of cell death and has been linked to the opening of MPTP (3–5). To examine whether the preservation of $\Delta\Psi_m$ is associated with the cardioprotective effects of SFA, we assessed the change of TMRE fluorescence by H_2O_2 stimulation in each group with FACS analysis. The majority of cells in the control group (Fig. 2A, panel C) belonged to a population with a high TMRE fluorescence level (indicated by vertical dashed line). Exposure to H_2O_2 shifted the predominant population to a lower TMRE fluorescence (Fig. 2A, panel H). Serofendic acid protected against the H_2O_2-induced loss of $\Delta\Psi_m$, preserving a population of cells with a normal $\Delta\Psi_m$ level (Fig. 2A, panel SFA). These observations were rendered quantitative by plotting the percentage of cells with high TMRE (>300, in this case), as shown in Figure 2B. Exposure to 100 μmol/l H_2O_2 for 1 h resulted in mitochondrial depolarization, whereas SFA prevented the loss of $\Delta\Psi_m$ in a concentration-dependent manner. The $\Delta\Psi_m$-preserving effect of SFA reached its maximum level at 100 μmol/l. We further compared the protective effects of SFA with those of diazoxide, a mitoK$_{ATP}$ channel opener. In isolated cardiac myocytes, we previously reported that diazoxide prevents the loss of $\Delta\Psi_m$ induced by oxidative stress in a concentration-dependent manner (8). As shown

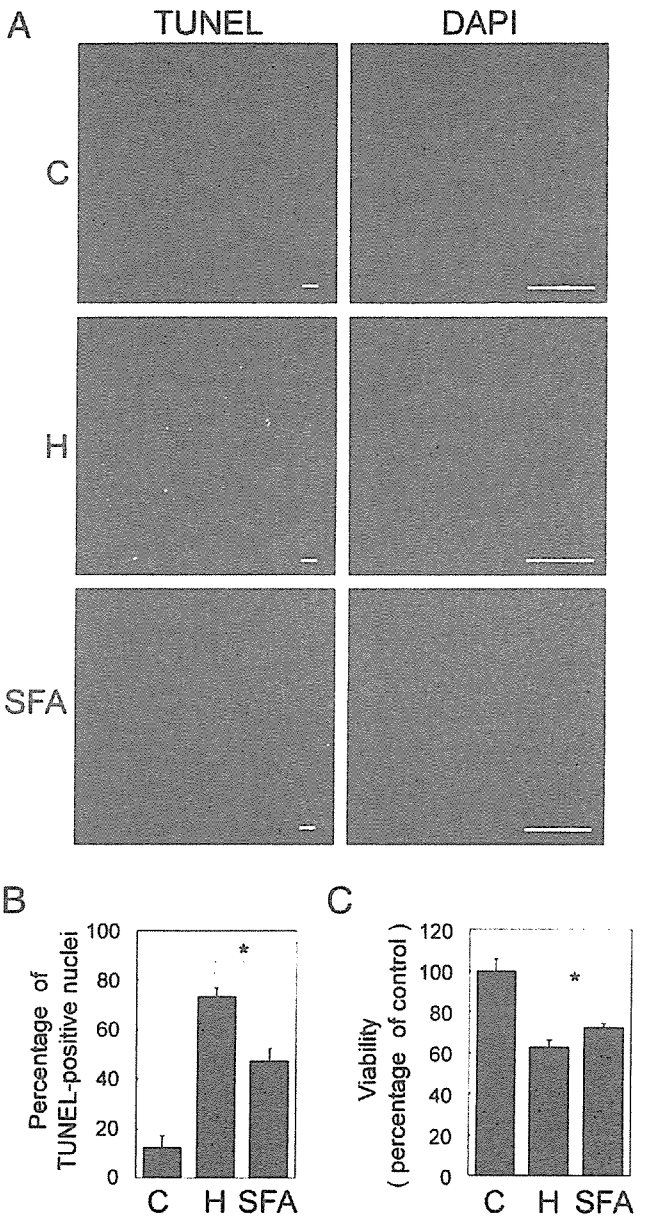

Figure 1. **(A)** The terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining **(left panels)** and the nuclear counterstaining by 4′,6-diamidino-2-phenylindole (DAPI) **(right panels)** in neonatal rat cardiac myocytes. C = control cells; H = cells exposed to 100 μmol/l H_2O_2 for 16 h; SFA = cells pretreated with 100 μmol/l serofendic acid for 30 min followed by 100 μmol/l H_2O_2 for 16 h. **Scale bars** = 20 μm. **(B)** Quantitative determination of TUNEL-positive nuclei (n = approximately 3 to 4 for each group from two independent cultures). An average of 200 to 400 nuclei from random fields was analyzed in each sample. **(C)** Cellular viability evaluated by MTS assay (n = 13 for each group from two independent cultures). The H_2O_2 treatment was 3 h in this experiment. *$p < 0.05$ versus group H.

in Figure 2C, the protective effect of 100 μmol/l SFA was comparable to 100 μmol/l diazoxide (maximal protective concentration of diazoxide [8]) in preventing the loss of $\Delta\Psi_m$ induced by 100 μmol/l H_2O_2. The protection afforded by SFA and diazoxide was completely blocked by a mitoK$_{ATP}$ channel blocker, 5-hydroxydecanoate (5-HD, 500 μmol/l [8]). The 5-HD alone did not aggravate the loss

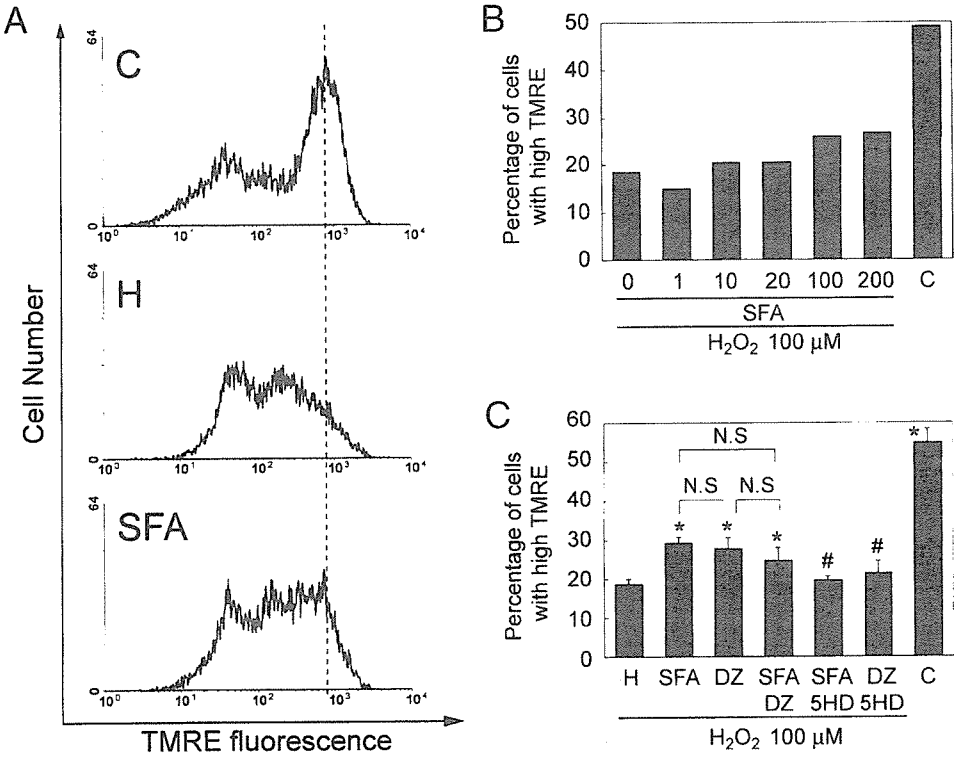

Figure 2. Mitochondrial inner membrane potential ($\Delta\Psi_m$) in neonatal rat cardiac myocytes. **(A)** FL-2 histograms of fluorescence-activated cell sorter data from tetramethylrhodamine ethyl ester (TMRE)-loaded cells are shown. H = cells exposed to 100 μmol/l H_2O_2 for 1 h; SFA = cells pretreated with 100 μmol/l SFA for 30 min followed by 100 μmol/l H_2O_2 for 1 h. In all of the histograms, the position of the major population of control group is indicated by a **vertical dashed line.** Results are representative data from at least three independent experiments. **(B)** Representative data of the percentage of cells that maintain high (>300) TMRE fluorescence. Cells were pretreated with various concentrations of SFA for 30 min, followed by 100 μmol/l H_2O_2 for 1 h. Serofendic acid preserved $\Delta\Psi_m$ in a concentration-dependent manner. **(C)** Summarized data of the percentage of cells that maintain high (>300) TMRE fluorescence. Cells were pretreated with various drugs for 30 min followed by 100 μmol/l H_2O_2 for 1 h. SFA = 100 μmol/l SFA; DZ = 100 μmol/l diazoxide; 5-HD = 500 μmol/l 5-hydroxydecanoate. *$p < 0.05$ versus H. #$p < 0.05$ versus corresponding 5-HD–absent group. Other abbreviations as in Figure 1.

of $\Delta\Psi_m$ elicited by 100 μmol/l H_2O_2 (data not shown). Co-application of 100 μmol/l diazoxide and 100 μmol/l SFA did not exhibit an additive effect (Fig. 2C), because the combination of maximal protective concentrations of both did not exceed each single drug. In addition, SFA alone in the absence of H_2O_2 did not affect the control level of $\Delta\Psi_m$ (data not shown).

To further confirm the protective effect of SFA in preventing the loss of $\Delta\Psi_m$, we examined the time-dependent changes of $\Delta\Psi_m$ on a single-cell basis (Fig. 3). Time-lapse confocal analysis of cardiac myocytes loaded with TMRE was performed at 2-min intervals. Time-lapse scanning began immediately after the application of 50 μmol/l H_2O_2. At first, we confirmed that TMRE fluorescence did not change during the 60 min of observation in the control group (Fig. 3A, panels C). In contrast, cells treated with H_2O_2 progressively lost their red fluorescence intensity, indicating the irreversible loss of $\Delta\Psi_m$ (Fig. 3A, panels H). The TMRE fluorescence was remarkably preserved in the SFA-treated group (Fig. 3A, panels SFA). Twenty-five cells were randomly selected in each group, and the TMRE fluorescence intensity from each individual cell was plotted in Figure 3B. Serofendic acid not only decreased the number of cells undergoing the dissipation of $\Delta\Psi_m$ but also delayed the onset of $\Delta\Psi_m$ loss, whereas it did not change the duration of $\Delta\Psi_m$ loss in unprotected cells. Figure 3C shows the average of TMRE fluorescence intensity from 25 randomly selected cells in each group, indicating the significant protective effects of SFA.

Reactive oxygen species is one of the most important inducers of MPTP opening. To investigate whether the suppression of ROS production is associated with the protective effects of SFA, we assessed the change of DCF fluorescence by 50 μmol/l H_2O_2 stimulation in each group with time-lapse confocal microscopy (Fig. 4). Cells in the control group gradually decreased the DCF fluorescence intensity (Fig. 4A, panels C). In the H_2O_2 group, the DCF fluorescence started to increase progressively upon H_2O_2 application (Fig. 4A, panels H). Serofendic acid suppressed the increase in DCF fluorescence (Fig. 4A, panels SFA). Twenty-five cells were randomly selected from each group, and the DCF fluorescence intensity from each individual cell was plotted in Figure 4B. The DCF fluorescence intensity of individual cells progressively increased in the H_2O_2 group, but the SFA-treated group blunted the overall increase of DCF fluorescence compared with the H_2O_2 group. Figure 4C shows the average of DCF fluorescence intensity from 25 randomly selected cells in each group.