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ORIGINAL ARTICLE
Vascular Biology and Vascular Medicine

Smoking Promotes Subclinical Atherosclerosis in Apparently Healthy Men

– 2-Year Ultrasonographic Follow-up –

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Background: Smoking is a major risk factor for cardiovascular disease. Also, inflammatory activation and metabolic disorder are the mediators of smoking-induced atherosclerotic progression. The aim of the present study was to investigate whether current smoking and smoking cessation alter inflammatory or metabolic status and affect subclinical atherosclerosis in apparently healthy men.

Methods and Results: Classical risk factors and smoking habit were evaluated in 354 men who completed health examinations annually without any current medications. Carotid intima-media thickness (IMT) was followed for 27.1±4.5 months. At baseline, both maximum and mean IMT significantly changed during 2-year follow-up. They tended to increase along with progression of smoking habit, with significantly greater maximum IMT in current smokers compared with never smokers. Both maximum and mean IMT significantly changed during 2-year follow-up, and tended to increase with progression of smoking habit, with maximum IMT being greatest for current smokers. Past smokers tended to have greater IMT increase than never smokers. Among smoking habit and some atherosclerotic risk markers that showed significant correlation with maximum IMT increase, stepwise regression showed that smoking habit and serum low-density lipoprotein-cholesterol (LDL-C) level were the only independent predictors.

Conclusions: Significant 2-year progression of subclinical atherosclerosis was associated with continuous smoking and LDL-C. This was only partly moderated in past smokers despite complete reversal of inflammatory activation, suggesting another crucial factor for inhibiting accelerated progression of subclinical atherosclerosis in men. (*Circ J* 2012; **76:** 2884–2891)

Key Words: Inflammation; Intima-media thickness; Metabolic syndrome; Progression; Smoking cessation

Previous epidemiological studies had proposed numerous risk factors for cardiovascular disease (CVD), such as hypertension, diabetes, and hyperlipidemia,[1] all of which comprise metabolic syndrome (MetS).[2] Also, it has been reported that lower plasma adiponectin[3] is an independent risk factor for CVD.[4] Furthermore, serum high-sensitivity C-reactive protein (hs-CRP) level is recognized as an independent predictor of CVD,[1] and serum interleukin-6 (IL-6) level is associated with increased incidence of CVD,[5] implicating inflammatory responses in the incidence of CVD.

Meanwhile, smoking has also emerged as an important risk factor for CVD,[6] and the inflammatory responses as well as impairment of MetS are thought to be involved in the underlying mechanisms of atherosclerosis development,[7,8] the leading cause of CVD.[9] Therefore, in addition to recovery from MetS through reduction of body weight or salt intake,[3,6] smoking cessation is generally and strongly recommended in current anti-atherosclerotic lifestyle improvement.[6] The impact, however, of smoking cessation on reduction of atherosclerotic changes and, if so, which mechanism confers the improvement, is not fully identified.

Recently, non-invasive measurements of arterial intima-media thickness (IMT) have been widely used for assessment of subclinical arterial alterations, and have demonstrated that this is a predictor of CVD.[9,10] In addition, the association of traditional risk factors with IMT (mainly maximum IMT) has been well examined.[11–13]

In the present study, to elucidate whether smoking cessation reduces or reverses the progression of atherosclerosis, and to explore what underlying mechanisms might be associated with

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Table 1. Baseline Clinical Subject Characteristics

	All	Smoking habit			P-value
		Never	Past	Current	
n	354	195 (55)	84 (24)	75 (21)	
Age (years)	48.5±5.7	47.7±5.6	49.6±5.7	49.4±5.5	0.009
BMI (kg/m²)	23.2±2.8	23.1±2.7	23.0±2.4	23.8±3.3	0.09
Waist (cm)	82.6±7.7	81.9±7.7	82.1±6.5	85.3±8.6	0.004
SBP (mmHg)	122±14	121±13	122±15	123±15	0.42
DBP (mmHg)	79±11	79±10	80±12	80±12	0.52
UA (mg/dl)	6.0±1.2	6.0±1.1	6.2±1.1	5.9±1.3	0.25
TG (mg/dl)	118±104	110±81	109±62	147±172	0.02
HDL-C (mg/dl)	56±14	57±15	57±12	55±14	0.74
LDL-C (mg/dl)	128±29	126±27	129±33	130±28	0.68
FPG (mg/dl)	92±12	91±12	94±11	90±10	0.18
HbA_{1c}, %	5.0±0.5	5.0±0.5	5.0±0.5	5.0±0.4	0.68
Max. IMT (mm)	0.922±0.502	0.877±0.471	0.958±0.474	1.001±0.597	0.15
Mean IMT (mm)	0.682±0.170	0.662±0.156	0.712±0.198	0.699±0.168	0.05
Presence of plaque	38 (10.7)	16 (8.2)	12 (14.3)	10 (13.3)	0.23

Data given as n (%) or mean±SD.
BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; IMT, intima-media thickness; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglycerides; UA, uric acid.

this reduction, we evaluated the associations of MetS parameters as well as inflammatory markers with IMT and their relationship with smoking habit in drug-naïve apparently healthy subjects.

Methods

Subjects

The subjects were the men who underwent health examinations in the Osaka University Health Care Center during 2005–2007. Apparently healthy Japanese men (n=354), 40–59 years of age, who completed an annual visit for medical checkup in 3 consecutive years, did not take any chronic or frequent medicine from at least 1 year before the first visit to the end of follow-up, did not suffer acute illness within 2 weeks before each visit and successfully underwent carotid ultrasonography in the first and the third visits were consecutively included. Informed consent was obtained from all subjects prior to participation in the study following approval of the study by the Ethics Committee of Osaka University. Because blood tests for hs-CRP, IL-6, and adiponectin concentration were beyond routine annual medical checkup, these tests were also performed in samples from 89 men (42/29/18 in never, past and current smokers, respectively) who participated in this study and who also agreed in writing to additional investigational measurements.

Definition of Past Smoker and Smoking Cessation Period

Smoking habit for each participant was primarily obtained from the mark in the check boxes sorting them into never, current or past smokers, as well as complementary descriptions determining the duration of smoking period in the interview sheet at annual medical checkup. For the past smokers, because the smoking cessation periods were not directly queried on the interview sheet, all the past interview sheet records for each individual were surveyed and the duration of smoking cessation defined as the period starting from the first year after the smoking habit changed from current to past smoker. If it was the case that all the past records indicated past smoking habit or the record was not available, we then referred to a formula of [(Age, years old)−(duration of smoking period, years)−20] year(s), based on the directly acquired data via the interview, to estimate the smoking cessation period.

Table 2. Smoking Status

	Past smoker, n (%)	Current smoker, n (%)
Smoking period (years)		
1–5	20 (23.8)	0 (0)
6–10	24 (28.6)	2 (2.7)
11–20	25 (29.8)	15 (20.3)
21–	15 (17.9)	57 (77.0)
No. cigarettes		
1–10	23 (34.3)	14 (18.7)
11–20	29 (43.3)	31 (41.3)
21–40	13 (19.4)	29 (38.7)
41–	2 (3.0)	1 (1.3)
Smoking cessation period (years)		
2–5	1 (1.2)	
6–10	12 (14.3)	
11–20	33 (39.3)	
21–30	27 (32.1)	
31–	11 (13.1)	

Risk Factor Assessment

Information on medical history, use of medicines and personal smoking habit were obtained via questionnaire, and was reconfirmed in expert interview by trained nurses. Waist circumference at the umbilical level was measured in the late exhalation phase in standing position.

Laboratory Measurements

Serum was collected from subjects after overnight fasting and kept at ≤−20°C until assay. Serum hs-CRP, IL-6 and adipo-

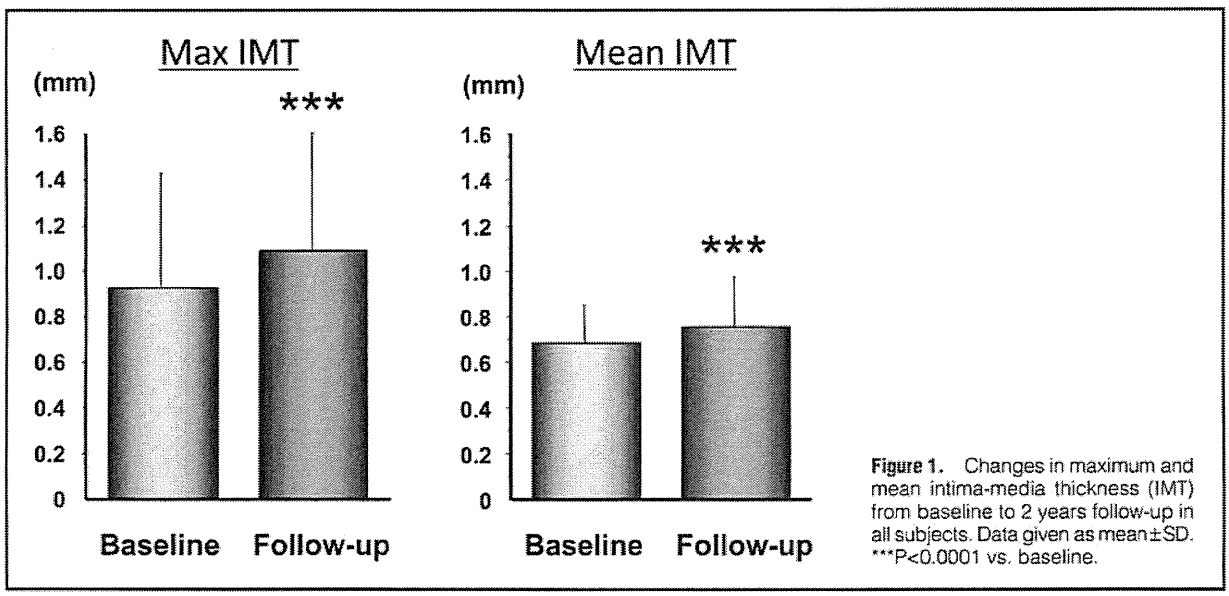

Figure 1. Changes in maximum and mean intima-media thickness (IMT) from baseline to 2 years follow-up in all subjects. Data given as mean±SD. ***P<0.0001 vs. baseline.

Table 3. Risk Factors and 2-Year Increase of IMT

	Delta-maximum IMT		Delta-mean IMT	
	r	P-value	r	P-value
Age	0.041	0.44	0.063	0.24
BMI	0.035	0.51	0.023	0.67
Waist	0.042	0.43	0.013	0.80
SBP	0.114	0.031	0.097	0.068
DBP	0.025	0.64	0.073	0.17
UA	0.079	0.14	0.110	0.039
TG	0.108	0.042	0.019	0.72
HDL-C	−0.029	0.58	0.052	0.33
LDL-C	0.142	0.009	0.155	0.004
FPG	0.087	0.10	0.092	0.082
HbA_{1c}	0.108	0.042	0.118	0.026
Smoking habit	0.130	0.015	0.096	0.071

Abbreviations as in Table 1.

nectin concentration were measured as described previously.[14,15] Briefly, they were measured using an immunoenzyme assay, a chemiluminescent enzyme immunoassay (CLEIA) and a sandwich enzyme-linked immunosorbent assay (ELISA) system, respectively.

The mean interclass coefficient of variation (CV) of hs-CRP, IL-6, and adiponectin measurements (n=40) in the assays before this study were 1.1%, 4.5%, and 1.2%, respectively. Kits from the same lots were used in this study to maintain reliability of measurement.

Evaluation of Carotid Atherosclerosis

All ultrasound examinations were performed by a single well-trained sonographer (K.I.) who regularly participated in quality control measurement sessions and was totally blinded to all clinical information, using LOGIQ 5 (GE Yokogawa Medical Systems, Tokyo, Japan) with an 8.8-MHz linear transducer. Three different longitudinal images (anterior oblique, lateral, and posterior oblique) of the left common carotid artery (CAA) of a 1.0–1.5-cm section at the distal end of the CCA proximal to the carotid bulb were obtained as described previously,[14,15] complying with validated protocols. In addition, transverse images were then obtained to confirm the accuracy of longitudinal images. After examination, the best longitudinal images were analyzed for each individual. Maximum and mean IMT was obtained using computer software that automatically traces the intima-media edge of the far wall. The presence of plaque was defined as detection of a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT or having a thickness of ≥1.5 mm, in concordance with a previous report.[16]

Statistical Analysis

Data were analyzed using SPSS 14.0 (Chicago, IL, USA). Pearson's correlation coefficients were calculated for variables with skewed distribution after logarithmic transformation. Stepwise multiple regression analysis was conducted using the enter method. ANOVA with modified Bonferroni's post-hoc test was used to assess differences between groups based on category. In order to analyze correlation of smoking with the progression of IMT, current, past and never smokers were scored as 1, 0.5 and 0, respectively, and the sum of this score was used, together with IMT progression within 2 years in each individual. P<0.05 was considered statistically significant.

Results

Baseline Demographics

Clinical characteristics of the study subjects are summarized in Table 1. With regard to risk factors, age was significantly older, and waist circumference and serum triglyceride (TG) level significantly higher as smoking habit progressed in men, whereas no significant differences were seen in body mass index (BMI), blood pressure (BP), uric acid (UA), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG) and HbA_{1c}. As shown in Table 1, both maximum and mean IMT tended to increase as smoking habit progressed, reaching significance in mean IMT. Plaques as defined in a previous report[16] were

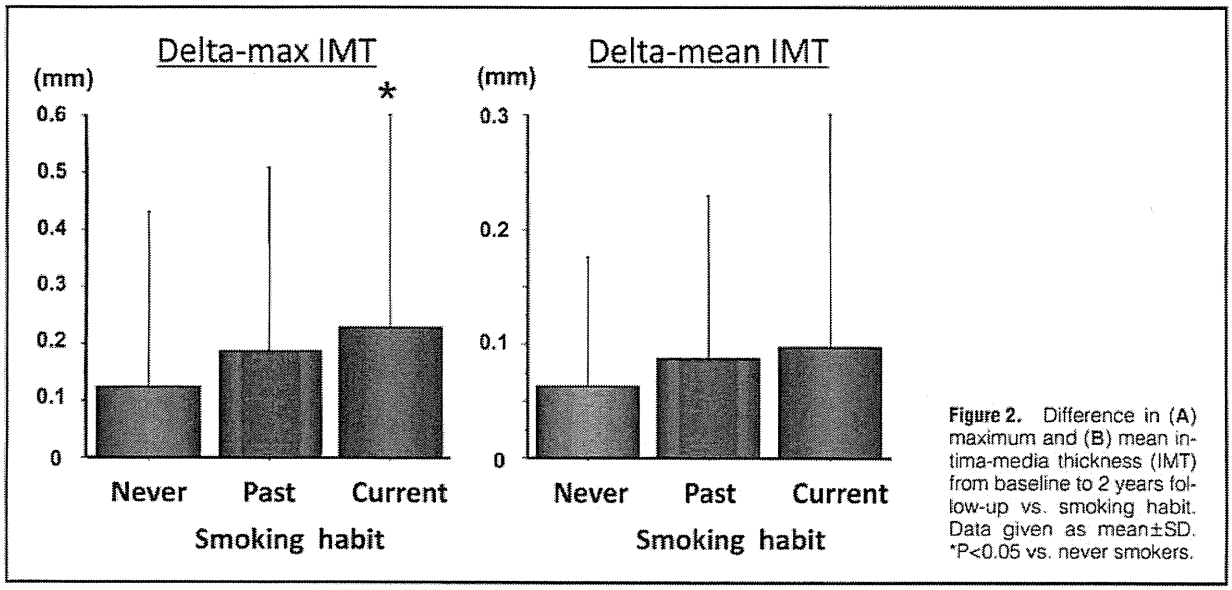

Figure 2. Difference in (A) maximum and (B) mean intima-media thickness (IMT) from baseline to 2 years follow-up vs. smoking habit. Data given as mean±SD. *P<0.05 vs. never smokers.

found in only 38 out of 355 individuals in the cohort (10.7%), without significant association with smoking habit.

Factors of smoking habit such as duration of smoking or number of cigarettes per day in current and past smokers are listed in Table 2. Current smokers were liable to have a longer smoking history, but there was no significant change in the number of cigarettes, and there were very few heavy smokers consuming >40 cigarettes per day in each group.

Progression of IMT During Follow-up and Association With MetS Components

After 2 years of follow-up, both maximum and mean IMT in all subjects significantly increased (P<0.0001) compared with baseline (Figure 1). Single regression analysis between the traditional risk factors and the change in IMT (delta-IMT) from before to after 2 years follow-up is given in Table 3. Among them, systolic BP, serum TG, LDL-C and HbA_{1c} as well as smoking habit were significantly correlated with delta-maximum IMT, whereas serum UA correlated only with delta-mean IMT, and serum LDL-C as well as HbA_{1c} were associated with both parameters. In contrast, age, BMI, waist circumference, diastolic BP, HDL-C and FPG were not correlated with delta-IMT.

Change in IMT During Follow-up and Association With Smoking Habit

Figure 2 charts delta-IMT during 2 years follow-up along with smoking habit (never, past and current smoker). Current smoking was associated with a tendency for increase in both delta-maximum and delta-mean IMT, which was significant in delta-maximum IMT. Past smokers tended to have less of an increase, and this did not reach significance. Among the aforedescribed MetS parameters as well as smoking habit, stepwise regression analysis (Table 4) showed that only smoking habit and serum LDL-C were significantly correlated with delta-maximum IMT, suggesting that these 2 parameters would be independent contributors for increased progression of atherosclerosis. Furthermore, to clarify whether MetS components had changed during the 2-year observation period, we additionally evaluated the relationship between IMT and the mean of the

Table 4. Independent Factors for IMT Increase in 2 Years

Independent variables	Delta-max. IMT		Delta-mean IMT	
	r	P-value	r	P-value
SBP	0.089	0.11		
UA			0.091	0.10
TG	−0.032	0.62		
LDL-C	0.119	0.036	0.115	0.040
HbA_{1c}	0.056	0.31	0.077	0.16
Smoking habit	0.111	0.041	0.099	0.082

Abbreviations as in Table 1.

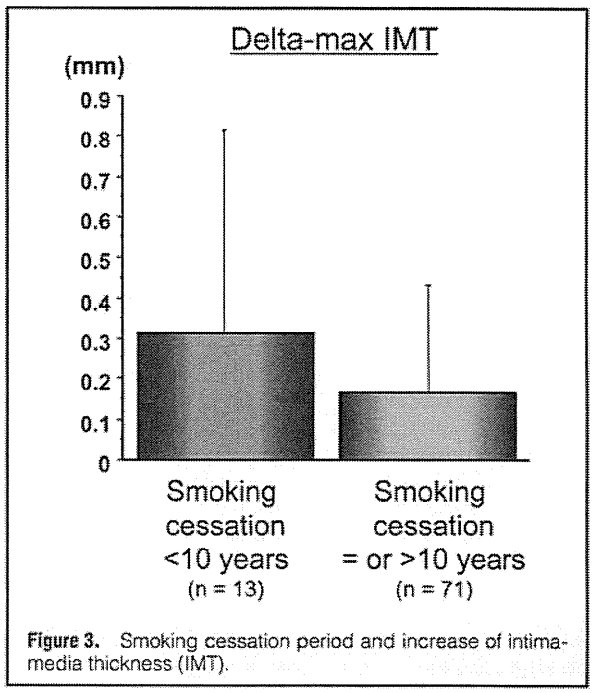

Figure 3. Smoking cessation period and increase of intima-media thickness (IMT).

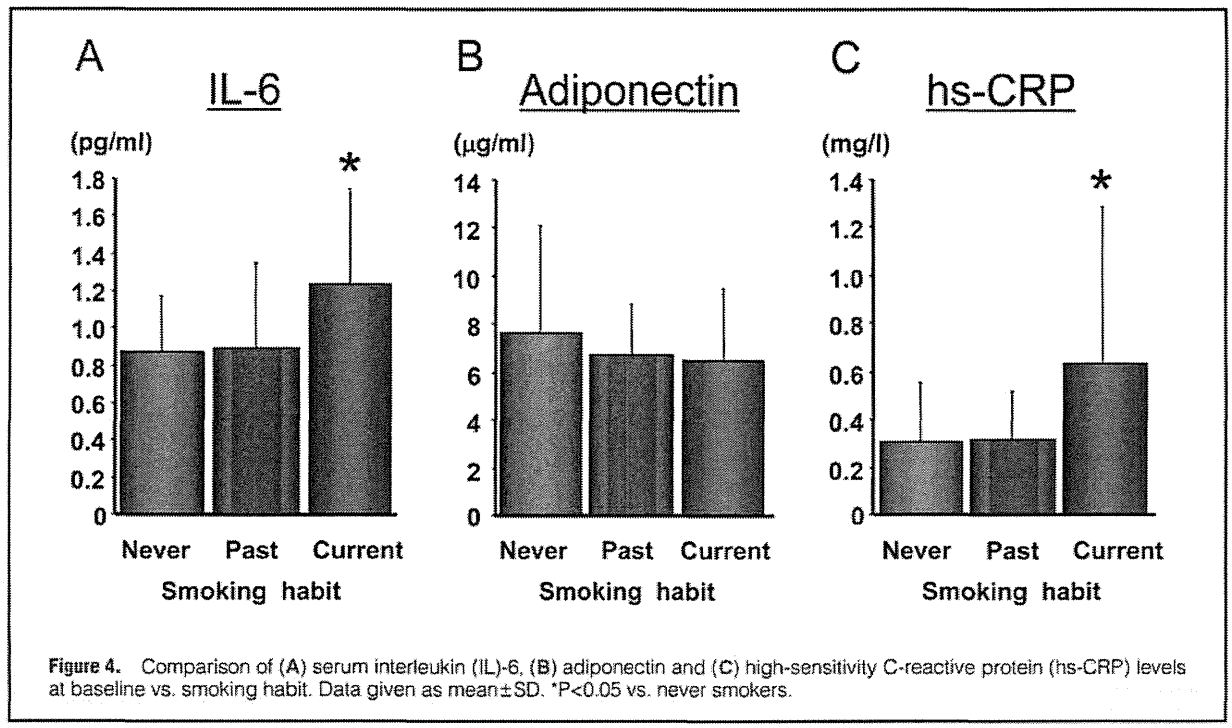

Figure 4. Comparison of (A) serum interleukin (IL)-6, (B) adiponectin and (C) high-sensitivity C-reactive protein (hs-CRP) levels at baseline vs. smoking habit. Data given as mean±SD. *P<0.05 vs. never smokers.

2-year blood test results (Table S1). We observed that only mean LDL-C during 2 years of follow-up had a significant association with progression of both maximum and mean IMT, further suggesting the important contribution of LDL-C to the progression of atherosclerosis. As to period of smoking cessation, longer estimated cessation >10 years tended to provide further moderation of progression of delta-maximum IMT (Figure 3), but was not significant (P=0.1139 and 0.534 in maximum and mean IMT, respectively). We then further investigated the association of atherosclerosis progression with either smoking or smoking cessation period, and found that positive correlation with smoking period might be stronger than the negative correlation with smoking cessation period among past smokers, although not significant (Table S2).

Smoking Habit, Serum IL-6, Adiponectin and hs-CRP

To further elucidate the underlying mechanisms, serum IL-6, adiponectin, and hs-CRP at baseline were evaluated. As shown in Figure 4, serum IL-6 and hs-CRP at baseline were significantly higher in current smokers (1.22±0.53 pg/ml and 0.63± 0.65 mg/L, respectively), compared with never and past smokers. Serum adiponectin tended to be higher in never smokers than past and current smokers, but no significant difference was observed. Furthermore, we found that IL-6 and hs-CRP had a significant positive correlation with the duration of smoking, and hs-CRP also had a significant negative correlation with duration of smoking cessation among past smokers (Table S3). Taken together, moderated progression of subclinical atherosclerosis was achieved in past smokers compared to current smokers, associated with complete reversal of inflammatory activation. This implies that smoking cessation and associated inflammatory deactivation might be another critical factor for inhibiting accelerated progression of subclinical atherosclerosis in addition to LDL-C lowering in men.

Discussion

In the present group of apparently healthy men we found that atherosclerosis, as evaluated via carotid IMT, significantly progressed, and was independently and significantly accelerated as serum LDL-C increased and as smoking habit progressed, as shown on multiple regression analysis. Serum hs-CRP and IL-6 were significantly higher in only current smokers, but in past smokers were completely the same as in never smokers.

Many previous studies have reported that smoking is a major promoter of atherosclerotic change,[17] and that cessation of smoking is strongly recommended, and is associated with possible reduction of risk for CVD.[18,19] Direct and quantitative evaluation of the impact of smoking cessation on long-term atherosclerotic change, however, was still to be documented. Here, in the present study, we have carried out a prospective 2-year follow-up of IMT changes in apparently healthy men. This had been done only in 1 previous report in healthy men/women,[19] which primarily supported the present findings of incomplete recovery from accelerated mean IMT increase after 2 years. The direct mechanistic linkage underlying smoking cessation and atherosclerotic progression, however, had not been investigated.

For the first time, we analyzed IMT change over time along with smoking habit, MetS status and multiple inflammatory parameters. We evaluated 89 samples from participants who agreed to additional blood sampling in writing, and on analysis of direct correlation between IL-6, hs-CRP and IMT, IL-6 had P=0.054 for positive correlation, whereas CRP did not have any association with IMT. Accordingly, our previous report evaluated this issue in a similar cohort of 153 apparently healthy men, and successfully observed the identical tendency.[15] Briefly, IL-6 was significantly correlated with either delta-maximum (P=0.02) or delta-mean (P=0.008) IMT, whereas the hs-CRP correlation was not significant (P=0.24 in delta-maximum

IMT and P=0.35 in delta-mean IMT). Therefore, we could assume that the inflammatory status represented by serum IL-6 potentially affects IMT progression. Although the NHANES III study (15,489 individuals) showed that blood CRP returned to baseline 5 years after smoking cessation,[20] which is consistent with the time frame associated with cardiovascular risk reduction observed in both the MONICA and Northwick Park Heart studies,[20–22] we intriguingly obtained the novel finding that the past smokers do not achieve complete recovery from accelerated increase in both maximum and mean IMT, despite complete reversal of inflammatory status after the shorter period of 2 years. This time frame-dependent dissociation might be explained in some way by the different characteristics and size of the subject group, but it may also be due to another long-lasting producer of IMT progression other than inflammatory status, that is, smoking. In this way, the underlying mechanisms of preclinical IMT progression and clinically relevant CVD incidence, as well as the markers representing the respective risks, should be somewhat different. Accordingly, regression analysis of the relationship between atherosclerosis progression and the duration of either smoking or smoking cessation period (**Tables S2,3**) showed that maximum-IMT had a stronger positive correlation with the duration of smoking period than negative correlation with the duration of smoking cessation period in past smokers, further supporting this idea. This might ultimately lead to the idea that interventions to avoid smoking from the beginning might be as important as those to stop smoking.

Because the inflammatory response is widely recognized as an independent risk factor for CVD,[1,5] and is reported to be closely associated with vulnerable plaque,[23] we can say that smoking cessation rapidly reduces the vulnerability of atherosclerotic regions via inflammatory inactivation, whereas more time, or even years, would be needed to reverse the acceleration of primary atherosclerotic progress represented by increased IMT due to other mechanisms. Accordingly, the Heinz Nixdorf Recall Study of 4,814 individuals without overt CHD also showed that the growth of the coronary atherosclerotic region is accelerated by smoking and slows down after smoking cessation, but advanced atherosclerotic change is present for a long period.[24] The question then arises as to what other mechanism than inflammatory response may be responsible for the prolonged IMT progression.

In this study the annual increase of mean IMT was around 0.030–0.050 mm, which might be greater than that reported in the many previous studies on the progression of IMT in healthy subjects. There are some reports, however, indicating that the progression of mean IMT in asymptomatic young adults varies from 0.015 to 0.029 mm/year.[25] Furthermore, we collected the present data from untreated and middle-aged individuals, who might be substantially more susceptible to IMT progression.[26] Taken together, we could say that the current data regarding the annual increase of mean IMT of around 0.030–0.050 mm might be within reasonable range.

As a possible mechanism, Oyama et al showed that green tea catechins have anti-atherosclerotic properties among smokers by increasing the level of nitric oxide and reducing oxidative stress.[27] In contrast, it is widely accepted that the impairment of MetS status substantially promotes the progression of atherosclerosis[28] and increases the risk of CVD.[1] Also, another study on a cohort of 5,033 individuals with the same characteristics as the present subjects suggested that the exposure to MetS would explain at least in part the increasing risk of excessive carotid plaque in past smokers.[29] The present result from stepwise regression analysis also showed that serum LDL-C level and smoking habit were the only independent predictors of IMT progression. This result is primarily supported by a previous study in a cohort of 2,421 individuals who have similar characteristics with the present subjects followed up for 14 years.[30] According to **Table 1**, however, past smokers have normal MetS parameters including BMI, waist circumference, BP, UA, TG, LDL-C and HDL-C and HbA_{1c}, which are equal to those of never smokers. This is possibly in part due to the relatively small size of the data set, because the data in **Table 2** differ from those in previous reports that suggested waist circumference[31] or HDL-C[32] as independent risk factors for accelerated atherosclerosis. Accordingly, the JART study in the same ethnic population showed that intensive lipid-lowering treatment with rosuvastatin effectively eliminated the progression of IMT compared with pravastatin treatment, and was associated with a much higher rate of achieving lower LDL-C/HDL-C ratio <1.5.[33] The present cohort had a mean LDL-C level within normal limits but a relatively higher LDL-C/HDL-C ratio (2.373±0.056 for never, 2.390±0.089 for past and 2.496±0.109 for current smokers, respectively, P=0.55), suggesting that more intensive lipid-lowering strategy beyond normalizing LDL-C level might facilitate the reduction of atherosclerosis progression. Otherwise, the present results might downgrade the relative importance of impaired MetS status including hypercholesterolemia as a putative promoter of smoking-induced prolonged atherosclerotic progression. The only difference we observed in past smokers compared with never smokers was a trend toward lower blood adiponectin level, which was almost identical to that of current smokers. Adiponectin is an adipocytokine mainly secreted from visceral fat tissues,[34] and the reduction of its blood level is reported to be an independent risk factor for atherosclerotic progression.[35] Although the duration for recovery of blood adiponectin level after smoking cessation is currently elusive, varying from 2 months to up to 20 years in men,[36,37] complete recovery from accelerated peripheral arterial atherosclerosis due to smoking represented by impaired ankle-brachial index will take up to 20 years after smoking cessation.[38] Experimental studies show that adiponectin has a direct cardioprotective property,[35] therefore adiponectin could be a potential contributor to smoking-induced prolonged atherosclerotic progression. Accordingly, **Table S3** suggests a potential inverse correlation of adiponectin level with smoking habit: decreasing with intensity and length of smoking ands increasing with the duration of smoking cessation, but it is possible that the 2-year follow-up period was too short to evaluate the long-term effect of adiponectin. This issue should be directly addressed by further study with an increased number of participants.

As a limitation, because smoking habit was confirmed only by questionnaire and interview, we cannot exclude the possibility that some of the past smokers were occasionally exposed to temporary smoking episodes during follow-up. Also, we did not follow the subjects for a longer period because of the study design. Furthermore, we did not measure luminal diameter routinely in this study. The primary aim of IMT measurement was to study the surrogate marker of atherosclerotic change in normal or preclinical stages. The core requirement to achieve this goal was to measure the initial and small changes of maximum and mean IMT precisely in a large cohort. To achieve this, we ensured that a well-trained and established sonographer performed the entire IMT test himself, to avoid inter-individual variance. This meant that it was necessary to limit the list of measurements to scoring of maximum and mean IMT, accompanied by informal observation of visually advanced narrowing, because IMT measurements were

performed on approximately 5,000 candidates and applicants every year, along with the institutional annual general medical checkup.

In addition, we could not measure the inflammatory markers (IL-6, adiponectin and hs-CRP) repeatedly because written informed consent limited the measurements to once only, at the time of entry.

Finally, we can conclude that subclinical atherosclerosis is independently accelerated via continuous smoking or LDL-C, and that the smoking-induced promotion of atherosclerotic change is closely associated with inflammatory reactions. Furthermore, the entire inhibition of activated inflammatory responses by smoking cessation is still insufficient to abrogate accelerated progression of subclinical atherosclerosis after 2 years in men, and reduced adiponectin level can be potentially proposed as an underlying mediator. Further investigation into the role of adiponectin in relation to smoking cessation in a larger cohort for a longer period is therefore warranted, to enable investigation of a direct mechanistic link between them, and to verify strategies to increase adiponectin as a therapeutic intervention against atherosclerosis and subsequent CVD events.

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Disclosures

Conflict of Interest: None.

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Supplementary Files

Supplementary File 1

Table S1. Correlation Between Mean Risk Factors During Study Period of 2 Years and Progression of IMT

Table S2. Correlation Between Smoking Habit and Progression of IMT in 2 Years

Table S3. Correlation Between Smoking Habit and Inflammatory Markers

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EDITORIAL

The Less Embraces the Greater in Detecting Multiple Coronary Artery Disease

Shoji Sanada, MD, PhD; Masafumi Kitakaze, MD, PhD; Issei Komuro, MD, PhD

Ischemic heart disease (IHD) is a major cause of mortality in both Western countries and Japan.[1] Recently, the morbidity and mortality of IHD have substantially improved because of progress in therapeutic strategies, represented by the dramatic evolution of percutaneous coronary intervention (PCI).[2] In fact, any sophisticated techniques in PCI are based on advanced imaging technologies to visualize the physical or structural causes of ischemia such as coronary stenosis or obstruction. However, as most cardiologists know, physical stenosis or obstruction does not necessarily reflect the incidence of myocardial ischemia in respective perfused regions within myocardium.[3]

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Myocardial ischemia ultimately originates from the mismatch in supply of energy substrates such as glucose, free fatty acids or oxygen in relation to myocardial energy demands within the cardiomyocytes;[1] however, in the majority of cases, it might be actually represented by a mismatch in local blood supply. Therefore, structural data on physical coronary stenosis or obstruction should be generally accompanied by information on regional "functional" shortage of blood supply to confirm regional myocardial ischemia. In that way, the somewhat conventional technology of single-photon emission computed tomography (SPECT), a semi-quantitative measurement of regional myocardial blood flow via blood flow-mediated myocardial incorporation of ions or metabolic substrates, is still widely accepted as a major and effective imaging strategy.[4] Primarily, it works best in detecting localized regional ischemia by prominently displaying low-flow areas in contrast with other good-flow areas by nature, and has some difficulty in precisely indicating multiple or global coronary ischemia.[5] One of the compensatory strategies for detecting such severe coronary artery disease (CAD) is to detect the stress-induced changes in dynamic cardiac performance, mainly by stress echocardiography.[6] Using this method, we can easily detect multiple or global coronary ischemia through a greater reduction in cardiac contractile performance upon stress.[7] ECG-based gated SPECT newly provides us with the additive information of dynamic changes in left ventricular chamber size and regional wall motion[5] that enable us to evaluate segmental blood flow and regional contractile function, namely physically and functionally active ischemia, at once and compare the integrated myocardial status with and without stress in a single modality.

Stress-induced ischemic changes in myocardial contractile performance mainly include transient postischemic myocardial stunning (PMS) and transient ischemic dilation (TID).[8] In this issue of the Journal, Hida et al[9] report on which phenomenon would better predict the existence of multiple coronary ischemia using 271 patients who underwent stress SPECT followed by coronary angiography (CAG). They found that PMS, defined as poststress increase in end-systolic volume (ESV) ≥5 ml, together with conventional criteria of multi-territorial ischemia in ATP-induced stress SPECT, best identified multiple CAD, with substantially high sensitivity (78%) and specificity (84%).

Intriguingly, although SPECT has been thought to be relatively weak in detecting broad ischemia, namely left main and/or 3-vessel disease,[5] the present study reports that this modality still tended to keep a satisfactory sensitivity of 74% and specificity of 57% with a poststress increase in ESV ≥5 ml, as well as those of 82% and 42%, respectively, with a poststress decrease in EF ≥2%, in detecting multiple coronary ischemia, whereas the TID ratio again turned out to be not significantly useful.[9] This finding has at least 2 notable aspects.

First, taken together with other sets of data that conventional diagnosis via multi-territorial malperfusion alone provides a similar sensitivity of 77%, but lower specificity of 55%, for predicting multiple coronary ischemia,[9] and that these values are not improved even in combination with an increased TID ratio,[9] the criteria proposed here (ie, PMS together with multi-territorial ischemia) has sufficient power of detecting broad and multiple CAD and might give substantial priority to stress SPECT test as a first-line extensive noninvasive examination following routine screening tests to diagnose multiple CAD in the clinical setting. In fact, invasive CAG is currently still serving as the best standard for detecting coronary structural stenosis because it can provide detailed and complicated information at once precisely, regardless of heart rate and arrhythmia. However, it is invasive and needs substantial X-ray exposure, as well as use of contrast medium. Recently, ECG-gated dynamic multidetector-row computed tomography has emerged as a possible alternative to CAG because of the striking progress in imaging and instrumental technology, but it also needs contrast medium, still has some restrictions in acquiring images against arrhythmia and tachycardia, and is

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weak in detecting accurate images around calcified regions, because of enhanced noise. Therefore, for patients with severe renal dysfunction or allergy to contrast medium who cannot choose these options, cardiac SPECT is one of a very few alternatives for the detection of myocardial functional ischemia, although it has another limitation of lacking images from the segments without live myocardium.

Second, this might give some clue to addressing the mechanistic consequences of ischemia-induced acute cardiac dysfunction. This study suggests that systolic dysfunction is more sensitive to ischemic stress than temporal dilation, implying that systolic dysfunction might be a primary reaction, leading to dilated changes and subsequent cardiac dysfunction.[8] This consequence is concomitant with the widely recognized idea from experimental animal studies,[10] also relevant in humans,[11] that myocardial stunning represented by immediate regional myocardial asystole is a primary reaction to acute ischemic stress, followed by cardiac bulging and dilation.[1] In that way, to detect regional reduced contraction immediately after acute ischemic stress might be quite a reasonable strategy of predicting multiple ischemic regions.

Although the accurate cutoff value should be further tested and adjusted in large-scale trials, this study potentiates the importance of gated SPECT as a quite helpful option for diagnosing multiple CAD in addition to contrast-based angiography. We hope that the present findings will help to diagnose and treat severe IHD more easily and feasibly, via an increased variety of modalities in real-world clinical medicine.

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Original article

Cardioprotective effects of low-dose combination therapy with a statin and an angiotensin receptor blocker in a rat myocardial infarction model

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KEYWORDS
Statin;
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Left ventricular systolic function;
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Summary
Purpose: Statins attenuate angiotensin II-induced myocyte hypertrophy and this might increase the cardioprotective effects of renin–angiotensin system inhibition in the ischemic heart. In this study, we investigated the cardioprotective effects of combination therapy with low-dose simvastatin and low-dose losartan using a rat myocardial infarction model.
Methods: Myocardial infarction was created in rats by left anterior descending artery ligation, and the animals were randomly allocated to one of four groups: control ($n=8$), losartan 3 mg/kg/day ($n=8$), simvastatin 2 mg/kg/day ($n=8$), and losartan 3 mg/kg/day plus simvastatin 2 mg/kg/day ($n=8$). Each treatment was started on the day of coronary ligation, and hemodynamics, myocardial blood flow, and infarct size were measured after 28 days.
Results: Blood pressure, heart rate, and left ventricular systolic and end-diastolic pressures were not significantly different comparing the control group with the 3 other treatment groups.

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The peak positive first derivative of left ventricular pressure (peak LV *dP/dt*) was equivalent comparing the control group with the losartan and simvastatin groups. However, the peak LV *dP/dt* was greater in the losartan plus simvastatin group than in the control group ($p < 0.05$). Myocardial blood flow, left ventricular weight, and infarct size were not significantly altered by the 3 treatments.

Conclusions: Treatment with 3 mg/kg/day losartan plus 2 mg/kg/day simvastatin but not losartan or simvastatin alone improved left ventricular systolic function in a rat myocardial infarction model. The result suggests that statins given in combination with angiotensin receptor blockers might have beneficial cardioprotective effects, even at low-doses for each agent.

Introduction

Blockade of the renin–angiotensin system (RAS) by angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) inhibits left ventricular hypertrophy and left ventricular remodeling, ameliorates left ventricular dysfunction [1], and consequently improves long-term prognosis [2] in patients with ischemic as well as non-ischemic heart failure.

The 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, statins, have been shown to reduce cardiovascular morbidity and mortality due to cholesterol-lowering effects [3]. However, other lipid-independent pleiotropic actions may contribute to cardioprotection induced by statins [4], and this has been documented in experimental studies demonstrating a decrease in oxidative stress, inflammation, inhibition of thrombogenic response, and atherosclerotic plaque formation [5] resulting in attenuation of vascular endothelial dysfunction. The mechanism responsible for the pleiotropic effects of statins involve the inhibition of small GTP-binding proteins, such as Ras, Rho, and Rac, which modulate a wide variety of cellular process [4] including the myocardial response to ischemic injury. We hypothesized that statins might have synergistic cardioprotective effects for renin–angiotensin system inhibition in the ischemic heart. Based on this hypothesis, in this study we investigated the effects of combination therapy with a statin (simvastatin) or an ARB (losartan) using a low-dose of both agents, which might not be effective, on cardiac function, coronary blood flow, and infarct size in a rat myocardial infarction model.

Methods

Study protocol

The left anterior descending coronary artery was ligated 2 mm below the left atrium with a 7–0 prolene suture in 32 adult (8-week-old) male Sprague-Dawley rats (191.9–230.6 g). Rats were randomly allocated to one of 4 treatment groups: control (0.5% CMC-Na^+ solution; $n = 8$), losartan 3 mg/kg/day ($n = 8$), simvastatin 2 mg/kg/day ($n = 8$), or losartan 3 mg/kg/day plus simvastatin 2 mg/kg/day ($n = 8$). Doses for oral administration were decided to be 10% of commonly used doses, 30 mg/kg/day and 20 mg/kg/day for losartan and simvastatin in the rat experiment, respectively, which yields plasma concentration similar to that seen in patients taking clinical doses. Both agents were suspended in 0.5% CMC-Na^+ solution and were given to rats by gastric gavage once a day. Each treatment was started on the day of onset of myocardial infarction by coronary ligation. Hemodynamic measurements were performed 28 days after the onset of myocardial infarction under barbiturate anesthesia and controlled ventilation. After the measurements, the anesthetized rats were euthanized with a lethal dose of barbiturate, and the hearts were removed to measure myocardial blood flow and infarct size.

Hemodynamic measurement

Under barbiturate anesthesia and controlled ventilation, a catheter was inserted into the right carotid artery to measure systolic (SBP) and diastolic blood pressure (DBP) and heart rate (HR). Then, the catheter tip was advanced into the left ventricle to record left ventricular systolic (LVSP) and end-diastolic pressures (LVEDP). Finally, left ventricular pressure was differentiated and the peak positive first derivative of left ventricular pressure (peak LV *dP/dt*) was measured.

Myocardial blood flow measurement

In 4 rats from each group, myocardial blood flow was measured using fluorescent microspheres. The microspheres were injected into the left ventricle through the catheter. Another catheter was inserted into the abdominal aorta via the femoral artery and a reference blood sample was withdrawn at a rate of 0.84 mL/min. After euthanasia, the left ventricle was removed and weighed. The left ventricular tissue was digested with 4 M potassium hydroxide containing 2% Tween 80. Then, the microspheres were filtered and dried, and dimethyl formamide was added to extract the dye in the solution. The solution was centrifuged at 4700 rpm for 5 min. Finally, the supernatant was collected to measure the absorbance and the optical density (OD) was calculated. The reference blood sample was similarly treated and myocardial blood flow was calculated using a standard formula [tissue OD × reference blood withdrawal rate/tissue weight (g) × reference blood OD].

Determination of infarct size

In 4 rats of each group, the infarct size was determined. After euthanasia, the whole heart was excised and the right and left ventricles were dissected. The weights of the whole heart, the right ventricle and the left ventricle were measured. Then, the left ventricle was sliced into 5

Table 1 Global parameters at the 28th day in each treatment group.

	Control ($n=8$)	Losartan ($n=8$)	Simvastatin ($n=8$)	Losartan + Simvastatin ($n=8$)
SBP (mmHg)	125 ± 7	127 ± 4	126 ± 5	141 ± 7
DBP (mmHg)	104 ± 5	105 ± 3	101 ± 3	112 ± 5
HR (/min)	403 ± 15	441 ± 6	406 ± 12	435 ± 16
LVSP (mmHg)	126 ± 5	129 ± 4	128 ± 6	126 ± 5
LVEDP (mmHg)	10.0 ± 1.1	6.8 ± 1.2	10.2 ± 2.5	6.9 ± 0.6
Peak LV dP/dt (mmHg/sec)	7229 ± 408	8263 ± 395	7375 ± 646	9071 ± 528*
Whole heart weight (g)	1.280 ± 0.036	1.322 ± 0.053	1.343 ± 0.066	1.281 ± 0.047
Ventricular weight (g)	1.122 ± 0.023	1.146 ± 0.036	1.155 ± 0.036	1.114 ± 0.030
Left ventricular weight (g)	0.798 ± 0.019	0.816 ± 0.016	0.806 ± 0.015	0.765 ± 0.021
Myocardial blood flow (mL/min/g)	3.87 ± 0.89 ($n=4$)	3.96 ± 0.54 ($n=4$)	3.79 ± 0.47 ($n=4$)	3.83 ± 0.73 ($n=4$)
Infarct size (%)	29.0 ± 1.6 ($n=4$)	29.8 ± 2.9 ($n=4$)	26.7 ± 3.5 ($n=4$)	27.4 ± 2.8 ($n=4$)

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure.
*$p < 0.05$ vs control.

transverse sections. After weighing each slice, 4 of the 5 sections were stained with 1% triphenyl tetrazolium chloride (TTC) in a phosphate buffer to detect infarcted tissue. The infarct area and left ventricular area were measured using specific image analysis software (WinROOF ver. 3.1; Mitani, Tokyo, Japan). The weight of the infarcted tissue in each section was calculated as the infarct area/left ventricular area × section weight. Infarct size (%) was calculated as the weight of all infarcted tissue/left ventricular weight.

Statistical analysis

Data are expressed as the mean ± SE. Normality of the distribution of variables was assessed using a Bartlett test. If the data were normally distributed, comparisons of each treatment group with the control group were performed using the parametric Dunnett test. If the data were not normally distributed, the groups were compared using a nonparametric Dunnett test. A value of $p < 0.05$ was considered to be statistically significant.

Results

All of the rats survived for 28 days after the onset of myocardial infarction. Global parameters measured on the 28th day of each treatment are shown in Table 1 and Fig. 1. Compared to control group treated with 0.5% CMC-Na^+ solution, SBP, DBP, HR, LVSP, and LVEDP were not significantly different in the groups treated with losartan alone, simvastatin alone, and losartan plus simvastatin (Table 1). The peak LV *dP/dt* was equivalent between the control group and the groups treated with losartan alone and simvastatin alone. However,

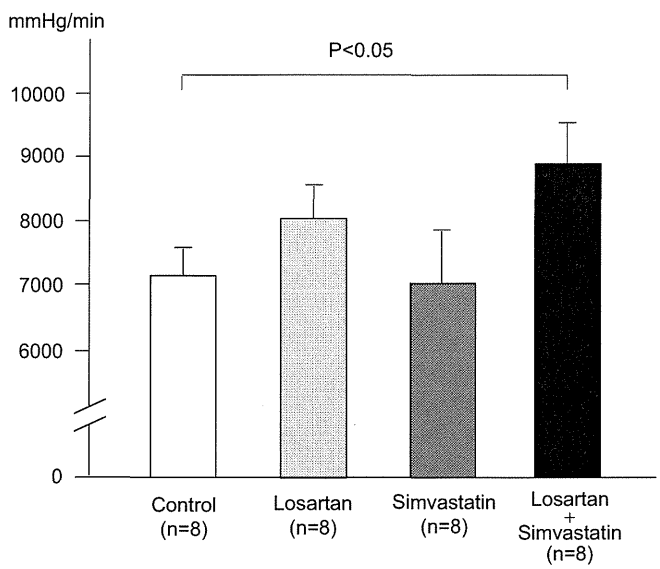

Figure 1 Peak positive first derivative of left ventricular pressure (peak LV *dP/dt*) at the 28th day in each treatment group. The peak LV *dP/dt* was similar in the groups of losartan alone and simvastatin alone to the control group but was greater in the losartan plus simvastatin group, compared to the control group.

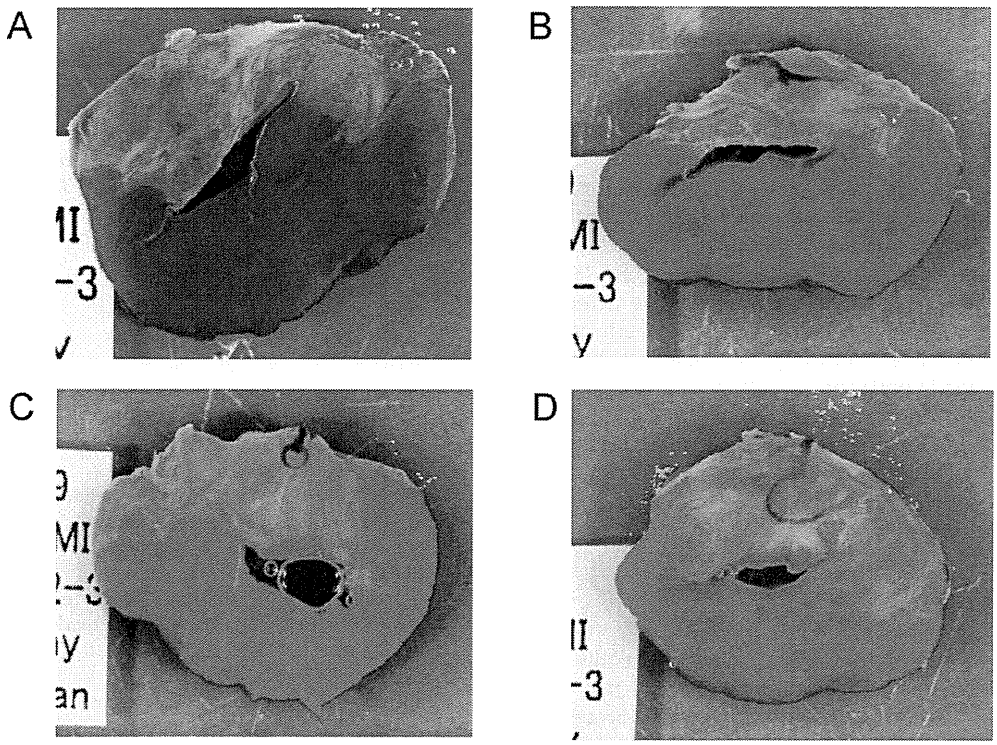

Figure 2 Infarct area in the sliced section at papillary muscle level. Infarct size was equivalent in each group of losartan alone (B), simvastatin alone (C) and losartan plus simvastatin (D) to the control group (A).

the peak LV *dP/dt* was significantly greater in the losartan plus simvastatin group than in the control group (9071 ± 528 vs 7229 ± 408 mmHg/s, $p < 0.05$) (Fig. 1). Myocardial blood flow was equivalent comparing the control group with the groups treated with losartan alone, simvastatin alone, or losartan plus simvastatin. Furthermore, the whole heart weight, ventricular weight, left ventricular weight, and infarct size (%) were also equivalent comparing the control group with the groups treated with losartan alone, simvastatin alone, or losartan plus simvastatin (Table 1 and Fig. 2).

Discussion

The major finding of our study is that treatment with losartan 3 mg/kg/day plus simvastatin 2 mg/kg/day for 28 days showed an improvement in peak LV *dP/dt* in a rat myocardial infarction model, although each monotherapy did not show such an effect. Doses of losartan and simvastatin were 10% of commonly used doses in the rat model, 30 mg/kg/day and 20 mg/kg/day, respectively, which yields plasma concentration corresponding to clinical doses. These results suggest that low-dose statins might have cardioprotective effects when given in combination with low-dose ARBs in the ischemic heart.

It has been postulated that inhibition of the renin-angiotensin system produces beneficial effects on cardiac function in ischemic as well as non-ischemic heart failure. ACEIs, ARBs, or both have been demonstrated to increase coronary blood flow and to reduce infarct size in canine myocardial ischemia models via a bradykinin-dependent mechanism [6]. Myocardial fibrosis is a major feature of left ventricular remodeling after myocardial infarction, which is mainly driven by angiotensin II [7,8]. In fact, ACEIs and ARBs have been shown to inhibit cardiac remodeling [8]. Furthermore, it has recently been demonstrated that statins also have cardioprotective as well as vascular protective effects that are independent of their lipid-lowering activity [9]. Acute and chronic statin treatment improves post-ischemic left ventricular contractile dysfunction in the isolated rat heart [10]. Statins also reduce infarct size in acute myocardial infarction or in an ischemia/reperfusion model [11]. Bao et al. demonstrated that the infarct size reduction by statins resulted from the pleiotropic effect represented by increased nitric oxide production [12]. Activation of phosphatidylinositol 3 kinase (PI3K)/Akt by statins may exert a preconditioning-like effect to limit infarct size [13]. In addition, statins may modulate left ventricular remodeling through effects on matrix metalloproteinases [14,15]. Improvement of cardiac function by short-term statin therapy has also been documented in the clinical setting [16,17].

Combined effects of statins and ARBs on cardiovascular systems have been previously reported. Lee et al. demonstrated additive effects of pravastatin and olmesartan on reduced left ventricular remodeling in rat myocardial infarction model [18]. Yamamoto et al. reported that pravastatin enhanced beneficial effects of olmesartan on vascular injury in salt-sensitive hypertensive rats [19]. In the present study, the low-dose treatment with losartan plus simvastatin did not alter SBP, DBP, LVSP, LVEDP, myocardial blood flow, left ventricular weight, and infarct size (%), but increased peak positive LV *dP/dt*. Therefore, the improvement in left

ventricular systolic function by losartan plus simvastatin might be independent of preload and afterload reduction, infarct size reduction, or inhibition of left ventricular remodeling, although peak positive LV *dP/dt* depends somewhat on preload and afterload. In our rat myocardial infarction model, 3 mg/kg/day losartan plus 2 mg/kg/day simvastatin but not the same dose of losartan or simvastatin alone improved left ventricular function. The result suggests that the doses of each agent might not solely influence hemodynamics but that the combination of ARBs and statins might have synergistic cardioprotective effects in myocardial ischemia, even at low-doses for both agents. Since statins have been shown to attenuate angiotensin II-induced myocyte hypertrophy in cultured neonatal rat cardiomyocytes in a manner that is probably mediated by the attenuation of oxidative stress [6], we can envision from our study that the pleiotropic, antioxidative effect of statins may potentiate the cardioprotective action of ARBs in the ischemic failing heart.

Study limitations

This study has several potential limitations. First this study did not include sham operated control data. In this study, myocardial infarction induction was performed through the left anterior descending artery ligation without following reperfusion, because we intended to exclude the impact of reperfusion injury. In most clinical settings, however, patients with acute myocardial infarction receive emergent reperfusion therapy with coronary angioplasty. So we might have to evaluate the pharmacological effects of statins plus ARBs on left ventricular function, using ischemia/reperfusion models. In addition, we did not measure serum lipid levels. To establish that a pleiotropic mechanism mediates the cardioprotective effects of statins when given in combination with ARBs, we should confirm that the cardioprotective effects are independent of lipid-lowering effects. In this study, we used peak positive LV *dP/dt* as a marker of left ventricular systolic function, but did not obtain data on peak negative LV *dP/dt* (*−dP/dt*), which is considered a marker of left ventricular diastolic function. Since diastolic function has recently been shown to be an important determinant of long-term prognosis in patients with chronic heart failure, the assessment of synergistic effects of ARBs and statins on diastolic function would be of interest. Finally, we did not investigate the mechanisms by which statins potentiate the effects of ARBs. The determination of the molecular basis of the cardioprotection will require additional experiments both in vivo and in vitro, especially focused on antioxidative effects.

Conclusion

The present study demonstrated that treatment with 3 mg/kg/day losartan plus 2 mg/kg/day simvastatin but not losartan or simvastatin alone improved left ventricular systolic function in a rat myocardial infarction model. The result suggests that statins given in combination with ARBs might have beneficial cardioprotective effects, even at low-doses for each agent.

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