

health states in a representative sample of the United Kingdom's general population, using the time trade-off (TTO) method.¹⁰ Based on these evaluations, utility scores can be deduced using an additive function. Utility scores may vary between -0.59 (worst health) and 1.00 (perfect health). In addition to the five dimensions, the EuroQol consists of an EQ-VAS ranging from 0 (worst imaginable health state) to 100 (best imaginable health state).¹¹ The HSS is a method of estimating patients' QOL, which we originally developed for the S-CATS trial. This score is calculated as the sum of scores (grades 1-5) of 10 hypertension-related symptoms (Figure 1).

The PSQI is a self-administered questionnaire to assess subjective sleep quality during the previous month.¹² The self-rated items of the PSQI generate seven component scores (range of subscale scores, 0-3) on sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping medications and daytime dysfunction. The sum of these seven component scores yields one global score of subjective sleep quality (range of scores, 0-21), with higher scores representing poorer subjective sleep quality. The psychometric properties of the PSQI have been confirmed in previous studies.^{12,13}

Data analysis

The data were collected before (baseline) and 3 months after the replacement of ARBs with the losartan/HCTZ combination pill therapy. The values were expressed as mean ± s.d. for parametric data and as median value and interquartile range for non-parametric data. The statistical analyses were performed using the paired *t*-test for parametric data and the Wilcoxon rank-sum test for non-parametric data. The correlation between the two variables was examined using the Spearman rank correlation coefficient. A *P*-value <0.05 was considered to be statistically significant.

RESULTS

We analyzed the data from 161 patients (72 men and 89 women, aged 70 ± 11 yrs) who were followed during the 3-month observation period. The characteristics of these patients are summarized in Table 1. There were no major adverse effects in this study. The baseline antihypertensive medications that the patients had been taking before

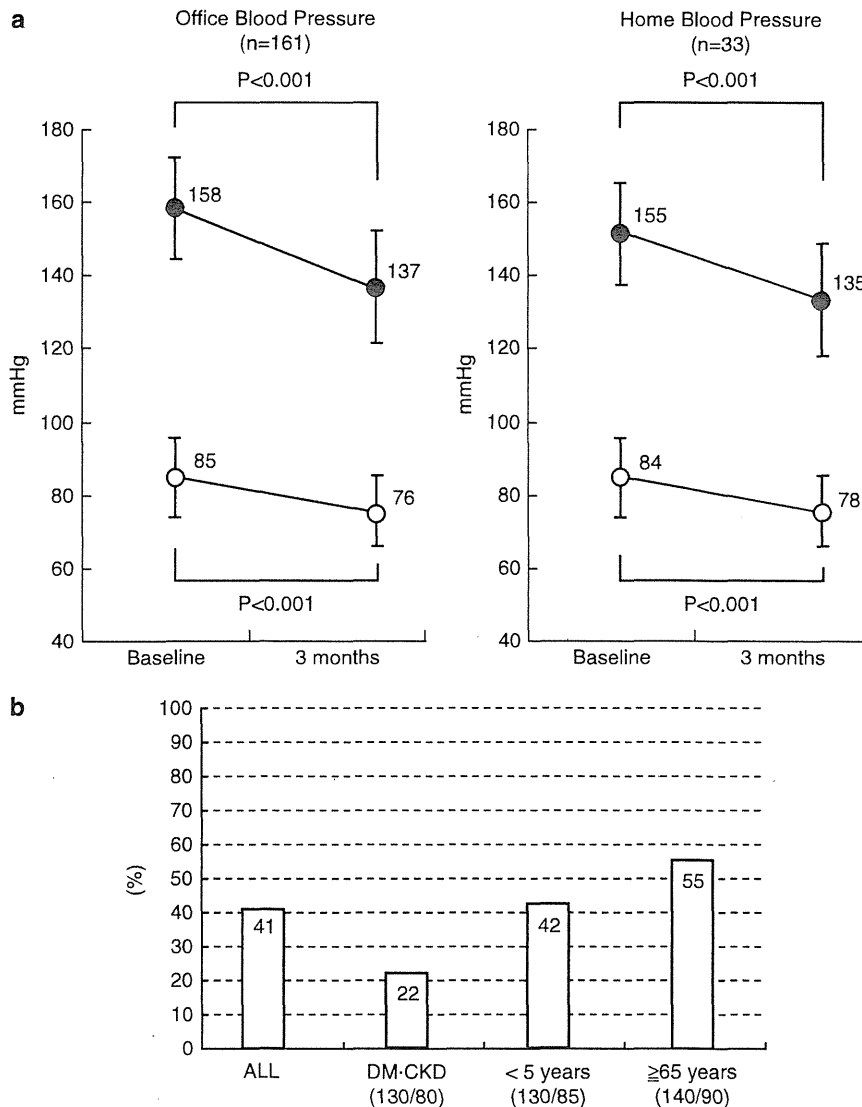


Figure 2 (a) Changes in blood pressure during the 3-month observation period after the replacement of ARBs with the losartan (50 mg)/HCTZ (12.5 mg) combination pill. After 3 months of treatment, the office blood pressures of all 161 patients decreased significantly (left). Home blood pressures also decreased significantly in 33 patients for whom the data were obtained both at baseline and 3 months after treatment with the combination pill (right). ● indicates systolic blood pressure, ○ indicates diastolic blood pressure (b) The guideline achievement rate shows the responders' blood pressure-lowering characteristics compared with those of the non-responders after changing the medication. CKD, chronic kidney disease; DM, diabetes mellitus.

the medication exchange were ARBs alone in 72 patients (45%) and ARBs with CCBs in 89 patients (55%). The baseline ARBs were losartan in 31 patients (19%), candesartan in 53 (33%), valsartan in 35 (22%), telmisartan in 24 (15%) and olmesartan in 18 (11%) (Table 2). During the 3-month observation period, office blood pressure decreased significantly (systolic 158 ± 14 to 137 ± 15 mmHg, $P < 0.001$; diastolic 85 ± 11 to 76 ± 10 mmHg, $P < 0.001$) (Figure 2a, left), and heart rate did not change significantly (73 ± 3 to 72 ± 3). Home blood pressure also decreased significantly (systolic 155 ± 21 to 135 ± 15 mmHg, $P < 0.001$; diastolic 84 ± 13 to 78 ± 11 mmHg, $P < 0.001$) (Figure 2a, right). As the result of adhering to the guidelines of the Japanese Society of Hypertension, there were difficulties in using the drug combination to treat the patients with complications of diabetic mellitus and chronic kidney disease (Figure 2b). The blood pressure-lowering effect was similar between the patients taking ARBs alone and those taking ARBs with CCBs as the baseline medications (Figure 3). In addition, the blood pressure-lowering effect was independent of the differences in baseline ARBs (Figure 4).

Changes in blood biochemistry profiles are shown in Table 1. Significant increases in serum levels of blood urea nitrogen (16.7 ± 4.1 to 18.7 ± 5.1 mg dl⁻¹, $P < 0.001$), uric acid (5.2 ± 1.4 to 5.5 ± 1.4 mg dl⁻¹, $P < 0.01$) and creatinine (0.77 ± 0.22 to

0.81 ± 0.24 mg dl⁻¹, $P < 0.001$) were evident during the observation period. Estimated glomerular filtration rate (eGFR) was calculated in 96 patients according to the Japanese Society of Nephrology Chronic Kidney Disease Practice Guide, and it was shown to decrease significantly from 70.9 ± 19.3 to 66.7 ± 17.5 ml min⁻¹ 1.73 m⁻² ($P < 0.001$). Of these 96 patients, 77 patients with an eGFR ≥ 50 ml min⁻¹ 1.73 m⁻² showed a significant decrease (76.9 ± 12.8 to 71.7 ± 12.2 , $P < 0.001$). In the remaining 19 patients with an eGFR < 50 ml min⁻¹ 1.73 m⁻², the eGFR value did not change significantly (46.7 ± 8.6 to 46.4 ± 10.7). Serum chloride and potassium levels decreased significantly (104 ± 3 to 103 ± 3 mEq l⁻¹, $P < 0.001$, 4.2 ± 0.4 to 4.1 ± 0.4 mEq l⁻¹, $P < 0.01$, respectively).

The EQ-5D index values analyzed in 95 patients increased significantly during the study from 1.00 (0.71–1.00) to 1.00 (0.76–1.00) ($P < 0.01$). Of these 95 patients, 20 patients with an EQ-5D < 0.7 showed a significant increase from 0.65 (0.59–0.69) to 0.67 (0.62–0.76) ($P < 0.05$). The score did not change significantly in the remaining 75 patients with an EQ-5D ≥ 0.7 (1.00 (0.77–1.00) to 1.00 (1.00–1.00)). The VAS analyzed in 96 patients increased significantly from 70.0 (68.8–80.0) to 80.0 (70.0–90.0) ($P < 0.01$). Of these 96 patients, 50 patients with a VAS < 70 showed a significant increase from 70.0 (56.3–70.0) to 70.0 (60.0–80.0), ($P < 0.001$). However, in the remaining 46 patients with a VAS ≥ 70 , the score did not change significantly (80.0 (80.0–90.0) to 85.0 (80.0–90.0)). The PSQI analyzed in 45 patients decreased significantly from 4.0 (2.0–7.0) to 3.0 (2.0–5.0) ($P < 0.05$). Of these 45 patients, 13 patients with a PSQI ≥ 5.5 showed a significant decrease (8.0 (7.0–9.0) to 7.0 (5.0–7.0), $P < 0.05$), whereas the score did not change significantly in the remaining 32 patients with a PSQI < 5.5 (3.0 (1.0–4.0) to 2.5 (1.0–4.0)) (Table 3). Finally, the HSS analyzed in 93 patients decreased significantly (4.0 (1.0–9.0) to 2.0 (1.0–8.0), $P < 0.05$) (Figure 5a). In these 93 patients, the change in HSS (the baseline value minus the 3-month value) correlated with systolic blood pressure ($R = 0.241$, $P = 0.0195$) (Figure 5b).

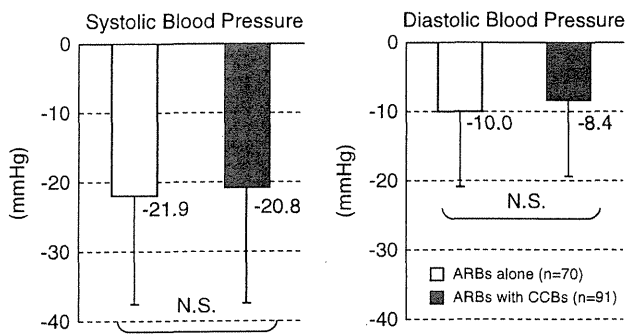


Figure 3 Comparison of the blood pressure-lowering effects between the patients taking ARBs alone and those taking ARBs with CCBs as the baseline medications. The effects were similar between two groups.

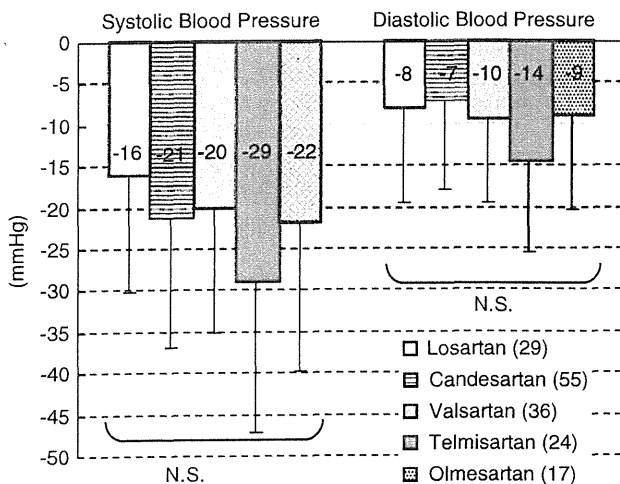


Figure 4 Comparison of blood pressure-lowering effects among the patient groups for each of baseline ARBs. The effects were similar among the groups.

DISCUSSION

In this study of hypertensive patients, the replacement of an ARB with a combination pill containing losartan and HCTZ achieved a mean reduction in systolic blood pressure of 21 mmHg and in diastolic blood pressure of 9 mmHg. In addition, all the QOL scores we

Table 3 Changes in quality of life (QOL) during the 3-month observation period

	n	Baseline	3 months	Value
<i>EuroQol 5 dimensions (EQ 5D)</i>				
	95	1.00 (0.71–1.00)	1.00 (0.76–1.00)	$P < 0.01$
< 7	20	0.65 (0.59–0.69)	0.67 (0.62–0.76)	$P < 0.05$
≥ 7	75	1.00 (0.77–1.00)	1.00 (1.00–1.00)	NS
<i>Visual analog scale (VAS)</i>				
	96	70.0 (68.8–80.0)	80.0 (70.0–90.0)	$P < 0.01$
< 70	50	70.0 (56.3–70.0)	70.0 (60.0–80.0)	$P < 0.001$
≥ 70	46	80.0 (80.0–90.0)	85.0 (80.0–90.0)	NS
<i>Pittsburgh sleep quality index (PSQI)</i>				
	45	4.0 (2.0–7.0)	3.0 (2.0–5.0)	$P < 0.05$
< 5.5	13	3.0 (1.0–4.0)	2.5 (1.0–4.0)	NS
≥ 5.5	32	8.0 (7.0–9.0)	7.0 (5.0–7.0)	$P < 0.05$

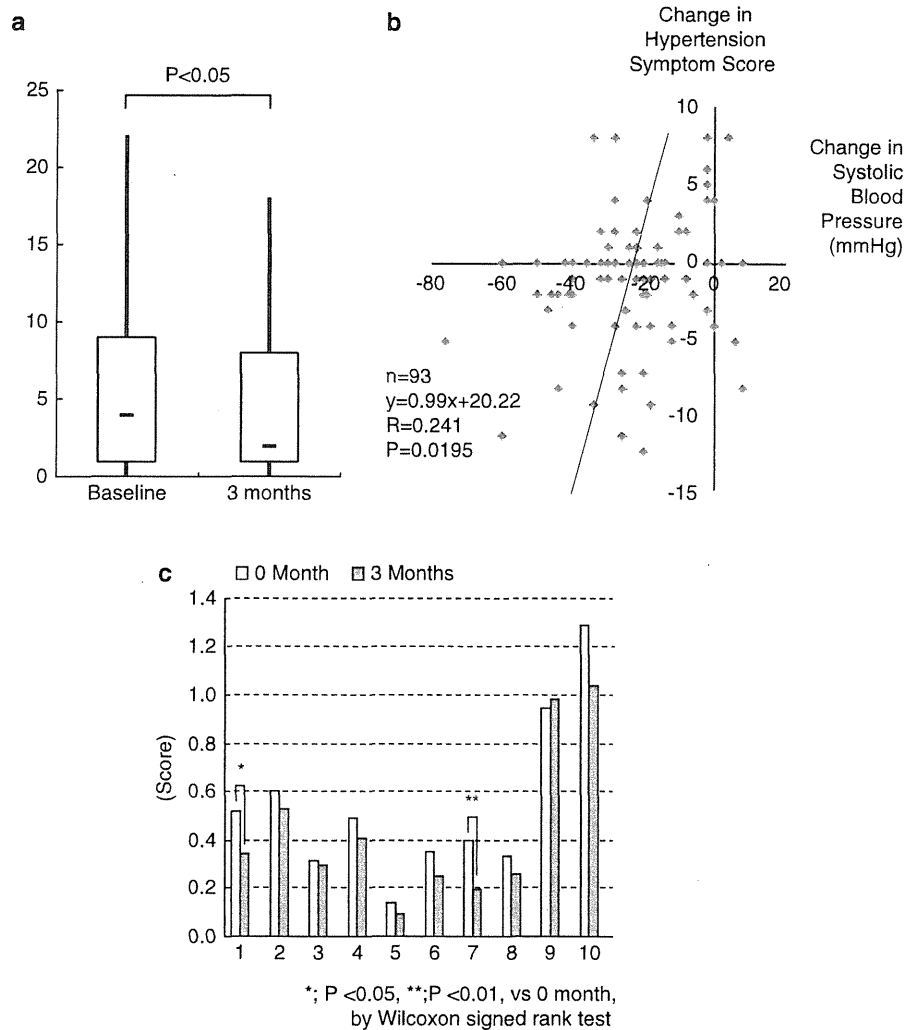


Figure 5 (a) Change in HSS after 3 months of treatment with the losartan (50 mg)/HCTZ (12.5 mg) combination pill ($n=93$). Overall, HSS decreased significantly (Wilcoxon rank-sum test). The solid line represents the median value; the box, the interquartile range; and the error bar, the 95% confidence interval. (b) Graph showing the positive correlation between the change in HSS and systolic blood pressure during the 3-month observation period following the replacement of ARBs with the losartan (50 mg)/HCTZ (12.5 mg) combination pill. (c) Self-assessment survey completed by patients with hypertension shows detailed examples of the effects of an improved hypertension symptom score. 1: headache or heaviness of head, 2: vertigo or tinnitus, 3: palpitation, 4: shortness of breath, 5: chest pain, 6: dizziness, 7: edema, 8: loss of concentration, 9: polyuria and 10: neck or shoulder stiffness.

measured in the study (that is EQ-5D, VAS, HSS and PSQI) improved after treatment with the losartan/HCTZ combination pill.

Patients seen in primary care settings often present with unspecific symptoms irrespective of the underlying medical problem. Many of these symptoms are non-specific. Patients with hypertension frequently report symptoms that are also reported by normotensive patients.¹⁴ Hypertension is usually described as asymptomatic in the absence of significant target organ damage and concomitant disease, although cognitive changes, mood alterations and general symptoms, such as dizziness and headache, have been described.^{15–19} Some symptoms are unique to the effects of antihypertensive drugs, whereas others overlap with symptoms described as or attributed to the hypertensive disease itself or are inseparable from those observed throughout the primary care population.¹⁴

The ultimate goal of antihypertensive treatment is the reduction of cardiovascular mortality and morbidity. To achieve this goal, treat-

ment compliance and medication adherence need to be maintained. If patients taking antihypertensive drugs do not feel a reduction in symptoms, it is unlikely that they will comply or adhere to the treatment. Symptoms, whether disease- or treatment-induced, may impair the health-related QOL of patients. QOL refers to the physical, emotional and social impact of a disease and its treatments^{20,21} and is distinct from the physiological measures of disease.^{20–23} Estimation of QOL may assess the impact of a disease and its treatment from a patient's perspective to a greater extent than it assesses conventional clinical symptoms. To estimate the patients' QOL, several scoring systems in the form of questionnaires on various health-related factors have been developed and validated. The EQ-5D is commonly used to measure health-related QOL and has been shown to be responsive, internally consistent and reliable in the normal population and other patient groups.^{9,10} The VAS is also used to measure health-related QOL. Although it is not as sensitive as other measures, the VAS does

have considerable merit because of its ease of application, which makes the collection of panel data feasible.¹¹ To date, there have been no reports that have specifically evaluated EQ-5D or VAS in the treatment of hypertension. However, another method for evaluating QOL, the 36-item Short Form questionnaire (SF-36), has been used in hypertensive patient populations. Because all these QOL scores represent health-related evaluations, we used our original QOL score from the HSS questionnaire in the present study. This questionnaire focuses on comparatively specific hypertension-related symptoms, calculated as the sum of five graded scores of 10 symptoms. We showed that the HSS score, as well as the EQ-5D and VAS scores, decreased after exchanging ARBs for the combination pill. In addition, the change in HSS score correlated significantly with the decrease in systolic blood pressure. Taken together, these results indicate that lowering blood pressure may improve QOL.

We also evaluated sleep quality with the PSQI during the course of treatment with the combination pill. This measurement showed that blood pressure lowering was associated with improved sleep quality, as shown by a decrease in PSQI. Sleep quality is one of the most important factors contributing to QOL. It has been reported that the prevalence of hypertension in subjects who are 'poor sleepers' is 87.1% compared with 35.1% in 'good sleepers'.²⁴ The increasing interest in the association between sleep disorders and significant comorbidities, including hypertension and glucose metabolism disorders, suggests that studies screening for cardiovascular risk should include an evaluation of sleep quality with questionnaires such as the PSQI. In this regard, our results indicate that the reduction in blood pressure caused by combined losartan/HCTZ treatment may lead to improvements in cardiovascular mortality and morbidity, partially as a consequence of improved sleep quality.

The results of our study also showed that the levels of blood urea nitrogen, uric acid and creatinine increased, whereas eGFR decreased during the 3 months of observation. These results may have been caused by transient decreases in intraglomerular pressure and a subsequent reduction in glomerular filtration, due to the blood pressure-lowering effect of the losartan/HCTZ combination pill. The changes observed in these renal function parameters, however, were within the normal range. In addition, decreases in eGFR were observed only in patients with a normal eGFR ($\geq 50 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$), whereas eGFR remained unchanged in patients with a low eGFR ($< 50 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$). These findings indicate that there are no safety problems associated with the use of the losartan/HCTZ combination pill. Simultaneous decreases in chloride and potassium levels, possibly caused by the effects of HCTZ, were also within the normal range. In this study, blood examination revealed increases in the plasma uric acid levels after 3 months. This increase might have been induced by the administration of HCTZ.

Potential limitations/clinical implications

The major limitation of the S-CATS study was an uncontrolled design involving a single-arm treatment in a relatively small number of patients. It is possible that confounding factors affected the present results. Therefore, we need to perform a two-armed randomized study in the future.

Our study included a limited number of patients because we could not obtain the informed consent from all the patients based on the QOL assessment. However, only practitioners of the Saga Medical Association performed this study and similar efforts by practitioners worldwide would be expected to result in improvements in cardio-

vascular morbidity and mortality among patients with hypertension. We therefore consider it essential that practitioners who are not specialists in the treatment of hypertension recognize the importance of adequately relieving patients' symptoms, thereby improving QOL and maintaining treatment compliance and medication adherence. On the basis of our results, we envisage that antihypertensive treatment with the losartan/HCTZ combination pill may result in a better long-term prognosis for patients with hypertension, partially as a consequence of improved QOL. In addition, we propose that the evaluation of QOL, including sleep quality, would be useful in the management of hypertension.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The following physicians in the Saga Medical Association, Japan, have made a significant contribution to this study (S-CATS): Hiroaki Kawano, Minekazu Hashimoto, Kazuo Moroe, Takahiko Imamura, Masanori Shida, Hiroyuki Tanaka, Genichirou Edakuni, Masao Kawahara, Taizou Minami, Shinichi Nakayama, Masanori Nishiyama, Reiko Yosioka, Hideyuki Kamochi, Norio Takeda, Michio Tomonaga, Fumihiko Saito, Mayumi Inoe, Toshifumi Uchida, Sadayoshi Fukuda, Akio Ikeda, Ryouta Kaihara, Katsuhiko Mizoguchi, Katsuya Oshima, Shouhei Sakai, Youichi Setoguchi, Shigeki Sugihara, Syungo Sukehiro, and Ken-ichi Tanaka. We are also thankful for the technical support provided by Sae Katafuchi and Aya Yamada. This study was supported by the Japan Heart Foundation.

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Relationships Among Hyperuricemia, Metabolic Syndrome, and Endothelial Function

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BACKGROUND

We evaluated the relationship of the severity of hyperuricemia and the flow-mediated vasodilatation of the brachial artery (FMD) in patients with and without the metabolic syndrome (MetS).

METHODS

In a cross-sectional study, FMD was obtained in 2,732 Japanese healthy men (49 ± 8 years) who had no cardiovascular (CV) disease and were not on any medication for CV risk factors. MetS was defined according to Japanese criteria, and serum uric acid (UA) levels in the upper half of the fifth (highest) quintile range were defined as severe hyperuricemia, whereas those in the lower half of this quintile range were defined as mild hyperuricemia.

RESULTS

Overall, the adjusted values of FMD were lower in the subjects with MetS (5.6 ± 0.1%; *n* = 413) than in those without MetS (6.2 ± 0.1%; *n* = 2,319) (*P* < 0.01). Among the subjects without MetS, the

adjusted values of FMD were lower in both the subgroups with mild hyperuricemia and severe hyperuricemia than in the subgroup without hyperuricemia. On the contrary, among the subjects with MetS, the adjusted value of FMD was lower only in the subgroup with severe hyperuricemia (4.8 ± 0.3%) as compared with that in the group without hyperuricemia (5.7 ± 0.2%) (*P* < 0.05).

CONCLUSIONS

In middle-aged healthy Japanese men without MetS, not only severe, but also mild hyperuricemia may be a significant independent risk factor for endothelial dysfunction in subjects without MetS, whereas only severe hyperuricemia (but not mild hyperuricemia) appeared to exacerbate endothelial dysfunction in similar subjects with MetS.

Keywords: blood pressure; endothelial function; hypertension; hyperuricemia; metabolic syndrome; risk factors; uric acid

American Journal of Hypertension, advance online publication 14 April 2011; doi:10.1038/ajh.2011.55

Recent studies have suggested that hyperuricemia is an independent risk factor for cardiovascular (CV) disease.^{1–4} The endothelium is a direct, sensitive target for the injurious effects of CV risk factors,⁵ and endothelial dysfunction, assessed by flow-mediated vasodilatation of the brachial artery caused by reactive hyperemia (FMD), is a marker of the early stages of atherosclerotic vascular damage.^{6,7} Some studies have indicated that the FMD is impaired in subjects with hyperuricemia.^{8,9} However, it still remains to be clarified whether not only severe hyperuricemia, but also mild hyperuricemia might

impair FMD. On the other hand, the metabolic syndrome (MetS), represents a clustering of risk factors of metabolic origin, is associated with an elevated risk of CV diseases,^{10,11} and FMD is also impaired in subjects with MetS.¹² Although hyperuricemia is common in subjects with MetS,¹³ it still remains to be clarified whether MetS and hyperuricemia might independently impair endothelial dysfunction.

The present cross-sectional study was conducted in a large cohort of middle-aged Japanese men who had no CV disease and were not on any medication for CV risk factors, including hyperuricemia, to evaluate the relationship between the severity of hyperuricemia and FMD in patients with and without MetS.

METHODS

Design and subjects. This cross-sectional study was conducted with the participation of three health care centers of companies and three health care clinics. All the participants were informed about the measurement of the FMD to examine its potential relationship with CV events. After providing written informed consent, the subjects underwent FMD measurement in addition to the routine annual health checkup, which included evaluation of atherosclerotic risk factors, between May and December 2008. A part of the data was reported

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Received 5 October 2010; first decision 27 November 2010; accepted 20 January 2011.

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elsewhere.⁷ The study protocol conformed to the principles of the Declaration of Helsinki (1964), and the study was conducted with the approval of the ethical guidelines committee of each of the participating institutions.

Assessment of FMD. The subjects were instructed to fast for at least 4 h, and to abstain from alcohol, smoking, caffeine and antioxidant vitamins for at least 12 h prior to the measurements. They were asked to rest in the sitting position in a quiet, dark, air-conditioned room (22–25 °C) for 5 min, followed by blood pressure measurement by the oscillometric method (UA 767; A&D Co. Ltd, Saitama, Japan). Then, after the subjects had rested again for at least 15 min in the supine position in the same room, the FMD measurement was conducted. We performed ultrasound measurements according to the guidelines for ultrasound assessment of FMD.¹⁴

Using high-resolution ultrasound with a 10-MHz linear array transducer, longitudinal images of the right brachial artery were recorded at the baseline and then continuously from 30 s before to 2 min or more after the cuff deflation following suprasystolic compression (50 mm Hg over the systolic blood pressure) of the right forearm for 5 min. The diastolic diameter of the brachial artery was determined semi-automatically using an instrument equipped with software for monitoring the brachial artery diameter (Unex Co. Ltd, Nagoya, Japan). In brief, continuous recordings of the two-dimensional gray-scale images and A-mode waves of the brachial artery in the longitudinal plane were conducted with a novel stereotactic probe-holding device. A segment with clear anterior (media-adventitia) and posterior (intima-media) interfaces was manually determined. These border interfaces were identified automatically on the A-mode waves as a signal of the intima-media complex, and the diastolic diameter of the brachial artery beat was synchronized with the electrocardiographic R-waves and tracked automatically. Changes in the diastolic diameter were continuously recorded. Then, FMD was estimated as the percent change of the diameter of the brachial artery over the baseline value at maximal dilatation during reactive hyperemia.

The reproducibility of the FMD measurements at each institute was determined from the measurements conducted at the three company health care centers and the health care clinic supervised by the Tokyo Medical University (Tokyo, Japan) (Pearson's correlation coefficient of the FMD between visits 1 and 2 was 0.86, $P < 0.01$, and the coefficient of variation was 11.2%; $n = 39$); it was also confirmed in the health care clinic supervised by the Hiroshima University (Hiroshima, Japan) (Pearson's correlation coefficient of the FMD between visit 1 and visit 2 was 0.89, $P < 0.01$, where the coefficient of variation was determined to be 10.1%; $N = 20$).

Laboratory measurements. We collected blood samples from fasting subjects after an interval of at least 1 h following assessment of the arterial endothelial function. The serum triglyceride, high-density lipoprotein cholesterol, total cholesterol, creatinine and uric acid (UA) levels, and the fasting blood glucose were measured using enzymatic methods.

Definition of metabolic syndrome, mild hyperuricemia and severe hyperuricemia. We adopted the criteria of the Japanese Expert Committee on the Diagnosis and Classification of Metabolic Syndrome for the clinical diagnosis of MetS;¹⁵ namely, central obesity (waist circumference ≥ 85 cm for men), plus at least two of the following three criteria: dyslipidemia (hypertriglyceridemia (triglyceride ≥ 1.70 mmol/l) and/or low high-density lipoprotein cholesterol (high-density lipoprotein cholesterol < 1.03 mmol/l), elevated blood pressure (blood pressure $\geq 130/85$ mm Hg), and elevated plasma glucose (fasting blood glucose ≥ 6.11 mmol/l).

Serum UA levels in the upper half of the fifth (highest) quintile range were defined as severe hyperuricemia, whereas those in the lower half of this quintile range were defined as mild hyperuricemia.

Statistical analysis. The normality of the distribution of the variables was assessed by the Kolmogorov–Smirnov test. The Kolmogorov–Smirnov test demonstrated that none of the continuous variables showed normal distribution. The linearity between the serum UA levels and FMD was assessed by the calculation of Pearson's correlation coefficient, and the relationship between the two variables was found to be weak, although significant ($r = 0.07$, $P < 0.01$). Therefore, linear model analyses were not applied for assessment of the relationship between the two variables. The continuous variables are represented as median values (25th–75th percentile). The Mann–Whitney test was applied to evaluate the differences in FMD between subjects with and without MetS. For assessment of the differences in the status of each variable among the groups, the Kruskal–Wallis test for continuous variables and Pearson's χ^2 test for categorical variables were applied. Furthermore, for assessment of the differences in the adjusted values of FMD among the groups, a general linear model analysis-*post hoc* pairwise comparison model with adjustments by the simple contrast method was applied. In the assessment of the differences in FMD between the subjects with and without MetS, the covariates used for the adjustments included the age, smoking status, total cholesterol, and creatinine. In the assessment of the differences in FMD among the three subgroups of subjects classified based on the presence/absence of mild/severe hyperuricemia, the covariates used for the adjustments included the age, waist circumference, smoking status, mean blood pressure, total cholesterol, high-density lipoprotein cholesterol, triglyceride, fasting blood glucose, and creatinine. All the analyses were conducted using the SPSS software for Windows, version 11.0J (SPSS, Chicago, IL); P values < 0.05 were considered to denote statistical significance.

RESULTS

In total, 4,447 subjects were successfully enrolled for the FMD measurement in the aforementioned six institutes. Among them, the following subjects were excluded from the study: 324 who failed to provide blood samples for measurement of the conventional CV risk factors or serum UA levels, 80 who were under medication for CV diseases, 513 who were under

Table 1 | Clinical characteristics of all the study subjects

Variable	
Number	2,732
Age (years)	49 (43–54)
WC (cm)	83 (78–88)
BMI	23.2 (21.5–25.1)
Smoking (%)	1038 (38.0)
SBP (mm Hg)	125 (115–136)
DBP (mm Hg)	78 (70–86)
HR (beats/min)	62 (56–68)
TC (mg/dl)	206 (185–228)
HDL (mg/dl)	57 (48–67)
TG (mg/dl)	116 (83–162)
FBS (mg/dl)	97 (91–104)
UA (mg/dl)	6.1 (5.4–6.9)
Crnn (mg/dl)	0.86 (0.79–0.93)
mildHUA (%)	305 (11.2)
sevHUA (%)	291 (10.7)
MetS (%)	413 (15.1)
FMD (%)	5.9 (4.1–7.8)

BMI, body mass index; Crnn, serum creatinine; DBP, diastolic blood pressure; FBS, fasting blood sugar; FMD, flow-mediated vasodilatation of the brachial artery caused by reactive hyperemia; HDL, serum high-density lipoprotein cholesterol; HR, heart rate; MetS, number of subjects with metabolic syndrome alone; mildHUA, number of subjects with mild hyperuricemia (UA levels ≥ 425 – <461 $\mu\text{mol/l}$); sevHUA, number of subjects with severe hyperuricemia (serum UA levels ≥ 461 $\mu\text{mol/l}$); Smoking, number of current smokers; SBP, systolic blood pressure; TC, serum total cholesterol; TG, serum triglycerides; UA, serum uric acid; WC, waist circumference.

medication for CV risk factors, including hyperuricemia, 75 who were age <30 , and 723 who were women (i.e., a relatively small percentage of the study subjects). Finally, the data of 2,732 subjects (age 49 ± 8 years) were included for the analyses.

Table 1 shows the clinical characteristics of the entire study population. In the entire study population, 413 subjects were diagnosed as having MetS. In the serum UA levels in the entire study population (150–712 $\mu\text{mol/l}$), serum UA levels >425 $\mu\text{mol/l}$ but <461 $\mu\text{mol/l}$ (in the lower half of the fifth (highest) quintile range) were defined as mild hyperuricemia, and serum UA levels >461 $\mu\text{mol/l}$ (in the upper half of the fifth (highest) quintile range) were defined as severe hyperuricemia. Then, 305 of the subjects were classified as having mild hyperuricemia and 291 as having severe hyperuricemia. The prevalence of mild hyperuricemia (with MetS (number of subjects = 70/413: 16.9%) vs. without MetS (number of subjects = 235/2319: 10.1%)) and that of severe hyperuricemia (with MetS (number of subjects = 86/413: 20.8%) vs. without MetS (number of subjects = 205/2319: 8.8%)) were significantly higher in the subjects with MetS than in those without MetS ($P < 0.01$).

In the study population as a whole ($n = 2,732$), the Mann-Whitney test demonstrated that the crude value of FMD was different between the subjects with and without MetS. The general linear model analysis demonstrated that the

Table 2 | Clinical characteristics of the study subjects with and without metabolic syndrome

Variable	Non	mildHUA	sevHUA	P value
<i>In subjects without MetS (n = 2,319)</i>				
Number	1,879	235	205	
Age (years)	49 (43–54)	49 (42–52)	49 (43–53)	0.17
WC (cm)	82 (77–86)	83 (80–87)	84 (80–89)	<0.01
BMI	22.6 (21.0–24.3)	23.4 (22.0–25.0)	23.7 (22.1–25.8)	<0.01
Smoking (%)	699 (37.2)	76 (32.3)	85 (41.5)	0.14
SBP (mm Hg)	122 (113–133)	124 (116–133)	125 (115–136)	<0.05
DBP (mm Hg)	76 (68–84)	77 (72–85)	77 (71–87)	<0.01
HR (beats/min)	61 (55–67)	62 (57–68)	62 (56–68)	0.06
TC (mg/dl)	203 (182–225)	209 (189–231)	210 (191–230)	<0.01
HDL (mg/dl)	59 (49–69)	56 (48–67)	57 (49–66)	0.07
TG (mg/dl)	106 (77–139)	116 (87–165)	126 (93–168)	<0.01
FBS (mg/dl)	96 (90–101)	97 (91–102)	97 (92–104)	<0.01
UA (mg/dl)	5.8 (5.2–6.4)	7.3 (7.2–7.4)	8.1 (7.9–8.6)	<0.01
Crnn (mg/dl)	0.85 (0.78–0.91)	0.90 (0.81–0.99)	0.92 (0.83–1.00)	<0.01
FMD (%)	6.2 (4.3–8.0)	5.7 (3.9–7.4)	5.6 (4.0–7.7)	<0.05
<i>In subjects with MetS (n = 413)</i>				
Number	257	70	86	
Age (years)	50 (47–56)	50 (49–58)	49 (41–54)	<0.05
WC (cm)	90 (87–93)	89 (86–92)	91 (87–95)	<0.05
BMI	25.5 (24.1–27.3)	25.8 (24.1–27.0)	26.4 (25.0–28.3)	<0.05
Smoking (%)	111 (43.1)	33 (47.1)	34 (39.5)	0.63
SBP (mm Hg)	136 (129–142)	137 (130–145)	136 (130–145)	0.56
DBP (mm Hg)	85 (79–91)	87 (80–93)	87 (82–94)	0.16
HR (beats/min)	65 (59–73)	65 (60–72)	65 (59–74)	0.83
TC (mg/dl)	214 (190–239)	226 (203–245)	227 (213–246)	<0.01
HDL (mg/dl)	49 (42–58)	51 (44–57)	49 (41–57)	0.71
TG (mg/dl)	182 (145–242)	183 (155–237)	216 (172–270)	<0.01
FBS (mg/dl)	110 (99–119)	105 (96–115)	105 (96–116)	<0.05
UA (mg/dl)	6.0 (5.4–6.5)	7.3 (7.2–7.5)	8.1 (7.9–8.7)	<0.01
Crnn (mg/dl)	0.83 (0.76–0.91)	0.87 (0.80–0.93)	0.90 (0.82–1.00)	<0.01
FMD (%)	5.4 (3.6–7.4)	5.2 (3.6–7.2)	4.9 (3.2–7.0)	<0.05

BMI, body mass index; Crnn, serum creatinine; DBP, diastolic blood pressure; FBS, fasting blood sugar; FMD, flow-mediated vasodilatation of the brachial artery caused by reactive hyperemia; HDL, serum high-density lipoprotein cholesterol; HR, heart rate; MetS, metabolic syndrome; mildHUA, subjects with mild hyperuricemia (serum UA levels ≥ 425 $\mu\text{mol/l}$ but <461 $\mu\text{mol/l}$); non, subjects without hyperuricemia; SBP, systolic blood pressure; sevHUA, subjects with severe hyperuricemia (serum UA levels ≥ 461 $\mu\text{mol/l}$); Smoking, number of current smokers; TC, serum total cholesterol; TG, serum triglycerides; UA, serum uric acid; WC, waist circumference. P value was assessed by Kruskal-Wallis test for continuous variables and Pearson's χ^2 test for categorical variables.

adjusted values of FMD were lower in the subjects with MetS ($5.6 \pm 0.1\%$; $n = 413$) than in those without MetS ($6.2 \pm 0.1\%$; $n = 2,319$). Then, the differences of the FMD among three subgroups of subjects classified based on the presence/absence of

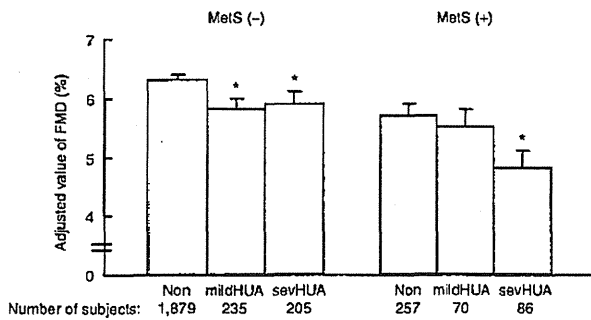


Figure 1 | The adjusted values of flow-mediated vasodilatation of the brachial artery (FMD) in the three groups of subjects classified based on the presence/absence of mild hyperuricemia/severe hyperuricemia in subjects with and without metabolic syndrome. MetS (+), with metabolic syndrome; MetS (-), without metabolic syndrome; mildHUA, subjects with mild hyperuricemia; non, subjects with neither mild hyperuricemia nor severe hyperuricemia; sevHUA, subjects with severe hyperuricemia. * $P < 0.05$ vs. the subjects with neither mild hyperuricemia nor severe hyperuricemia.

mild/severe hyperuricemia were examined separately in the subjects with and without MetS.

Table 2 shows the clinical characteristics of the three subgroups of subjects with/without MetS classified based on the presence/absence of mild/severe hyperuricemia. In both subjects with and without MetS, in addition to the FMD value, the waist circumference, total cholesterol, triglyceride, fasting blood glucose and creatinine also differed significantly among the three subgroups of subjects classified based on the presence/absence of mild/severe hyperuricemia.

The Kruskal-Wallis test demonstrated that the crude value of FMD was different among the subgroups with mild hyperuricemia, severe hyperuricemia and without hyperuricemia, not only in the subject population without MetS but also in that with MetS. As shown in Figure 1, the general linear model analysis with the simple contrast method demonstrated that in subjects without MetS ($n = 2,319$), the adjusted values of FMD in both the subgroups with mild hyperuricemia and severe hyperuricemia were lower than those in the subgroup without hyperuricemia. On the contrary, in the subjects with MetS ($n = 413$), the adjusted value of FMD was lower only in the subgroup with severe hyperuricemia (and not in the group with mild hyperuricemia) as compared with that in the subgroup without hyperuricemia.

DISCUSSION

Several studies have indicated that the conventional risk factors for CV disease are predictive of impaired FMD.^{6,7} Several studies have suggested that hyperuricemia is a causal factor for vascular damage.^{16,17} An experimental study demonstrated that UA impairs endothelial function via reduction of nitric oxide synthase.¹⁸ Although allopurinol has been shown to improve the FMD, it has not been clarified whether this effect is mediated by the reduction of the serum UA levels by the drug or reduction of xanthine oxidase activity by the drug.^{19,20} In addition, only studies with a relatively small number of subjects have reported the existence of a significant relationship

between the serum UA levels and the FMD.^{8,9} The present study, conducted on a large number of middle-aged healthy Japanese men, confirmed that not only severe hyperuricemia (serum UA levels $\geq 461 \mu\text{mol/l}$) but also mild hyperuricemia (serum UA levels $\geq 425 \mu\text{mol/l}$ but $<461 \mu\text{mol/l}$) impairs FMD, independent of the risk factors for CV disease, including the components of MetS.

Existence of a significant relationship between MetS and hyperuricemia has been reported.^{2,4} In this connection, the hyperinsulinemia associated with MetS has been shown to increase UA reabsorption from the proximal renal tubules, and the microvascular damage associated with elevated blood pressure has been shown to enhance UA generation.^{2,4} The present study demonstrated a higher prevalence of mild/severe hyperuricemia in the subjects with MetS than in those without MetS. Thus, clarification of the effects of hyperuricemia on the atherogenic abnormalities associated with MetS is crucial. It is noteworthy that the clustering of CV risk factors in MetS is associated with a greater risk for CV diseases than that reflected by the sum of the risks associated with the individual risk factors.^{10,11} The Framingham Offspring study demonstrated that the clustering of the CV risk factors in MetS is associated with progressive impairment of the FMD.¹² In the present study, different from the subjects without MetS, severe hyperuricemia, but not mild hyperuricemia, significantly impaired FMD. Thus, severe hyperuricemia may make a significant additional contribution to the progressive impairment of endothelial function associated with the clustering of CV risk factors in subjects with MetS.

Some prospective studies have demonstrated that hyperuricemia is a risk factor for CV events independent of the presence of MetS.^{4,13} On the other hand, hyperuricemia has also been reported to be a risk factor for increased carotid intima-media thickness, a morphological marker of atherosclerosis, as assessed by intravascular ultrasound examination.²¹ However, in two recent studies, hyperuricemia was not demonstrated as a potent risk factor for increased carotid intima-media thickness in subjects with MetS.^{22,23} As compared to the carotid intima-media thickness, impaired FMD is considered to be an earlier marker of atherosclerosis, preceding the appearance of ultrasonic evidence of atherosclerosis.²⁴ Several factors, such as conventional risk factors for CV disease, inflammation, oxidative stress, nitric oxide and so on, contribute to the initiation/progression of atherosclerosis.²⁴⁻²⁶ FMD is a marker related to endothelial nitric oxide bioavailability,^{25,26} and therefore, hyperuricemia may affect atherogenic abnormalities related to nitric oxide bioavailability in MetS.

This study had some limitations: (i) The linearity between the serum UA levels and the FMD was weak, although significant and therefore, the significance of the interaction of the effects of hyperuricemia and MetS on FMD was not assessed. (ii) The present study had some technical limitations related to the measurement of FMD;²⁷ (ii-A) Although FMD shows diurnal variations,²⁷ it was measured in the morning in some of the study subjects, but around noontime in others; (ii-B) Furthermore, the results of FMD measurement are operator

dependent. The reproducibility of FMD measurement at the participating study institutions is described in the Methods section, and for the present study, the FMD measurements were conducted using the same protocol and the same instrument. Even so, the consistency of FMD measurements among institutions was not examined in the present study. (iii) In the present study, abnormal glucose metabolism, a well-known risk factor for endothelial dysfunction, was not significantly related to the impairment of FMD. A plausible mechanisms to explain this unexpected result are that the status of abnormal glucose metabolism was not severe enough to impair FMD. (iv) The present study could not clarify the mechanisms underlying the impairment of endothelial function induced by hyperuricemia. (v) Gender-related differences in serum UA levels are widely recognized,^{1,10,13} and some studies have suggested that the relationship between hyperuricemia and MetS is relatively weak in males.^{22,23} Thus, any gender-related differences in the relationships among hyperuricemia, endothelial function, and MetS should be clarified in the future study. (vi) Although endothelium-independent vasodilatation is also a marker of vascular damage,²⁴ we did not evaluate the effect of hyperuricemia on endothelium-independent vasodilatation in the present study. (vii) In this multicenter study, although the FMD was assessed using the same protocol and the same instrument, the biochemical assays were not calibrated. Notwithstanding, however, the serum uric acid levels were similar among the clinics (data not shown). (viii) In the present study, the lipid profile was entered as a covariate for the analyses, and the National Cholesterol Education Program has recommended a fasting period of at least 8-h duration prior to its assessment.²⁸ However, in the present study, the lipid profile was determined after 5-h fasting in the subjects.

In middle-aged healthy Japanese men without MetS, not only severe, but also mild hyperuricemia may be a significant independent risk factor for endothelial dysfunction, whereas only severe hyperuricemia (but not mild hyperuricemia) appeared to exacerbate endothelial dysfunction in similar subjects with MetS.

Acknowledgment: This study was partially supported by grant in aid of Japanese Arteriosclerosis Prevention Fund.

Disclosure: The authors declared no conflict of interest.

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STATE-OF-THE-ART PAPER

Vascular Inflammation and Repair

CME

Implications for Re-Endothelialization, Restenosis, and Stent Thrombosis

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CME Editor Disclosure: *JACC: Cardiovascular Interventions* CME Editor Habib Samady, MB, ChB, FACC, has research grants from the Wallace H. Coulter Foundation, Volcano Corp., St. Jude Medical, Forrest Pharmaceuticals Inc., and Pfizer Inc.

Author Disclosure: This work was supported in part by grants from the National Heart, Lung, and Blood Institute to Dr. Simon (HL85816, HL57506 MERIT Award, HL73852) and to Dr. Croce (1K08HL086672); a Future Leaders in Cardiovascular Medicine Fellowship Grant to Dr. Croce; an award from the Michael Lerner Foundation to Dr. Croce; a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by a grant from Kimura Foundation to Drs. Inoue and Node; and by a research grant from the Japan Foundation of Cardiovascular Research to Drs. Inoue and Node. Dr. Simon is on the advisory board and is a consultant for Cordis/Johnson & Johnson and Medtronic Vascular; and is a consultant for Daiichi-Sankyo. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Medium of Participation: Print (article only); online (article and quiz).

CME Term of Approval:

Issue Date: October 2011

Expiration Date: September 30, 2012

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Vascular Inflammation and Repair

Implications for Re-Endothelialization, Restenosis, and Stent Thrombosis

The cellular and molecular processes that control vascular injury responses after percutaneous coronary intervention involve a complex interplay among vascular cells and progenitor cells that control arterial remodeling, neointimal proliferation, and re-endothelialization. Drug-eluting stents (DES) improve the efficacy of percutaneous coronary intervention by modulating vascular inflammation and preventing neointimal proliferation and restenosis. Although positive effects of DES reduce inflammation and restenosis, negative effects delay re-endothelialization and impair endothelial function. Delayed re-endothelialization and impaired endothelial function are linked to stent thrombosis and adverse clinical outcomes after DES use. Compared with bare-metal stents, DES also differentially modulate mobilization, homing, and differentiation of vascular progenitor cells involved in re-endothelialization and neointimal proliferation. The effects of DES on vascular inflammation and repair directly impact clinical outcomes with these devices and dictate requirements for extended-duration dual antiplatelet therapy. (J Am Coll Cardiol Intv 2011;4:1057-66) © 2011 by the American College of Cardiology Foundation

Drug-eluting stents (DES) substantially reduce angiographic and clinical restenosis by 70% across broad patient and lesion subsets and decrease repeat target lesion interventions. The prototypical antiproliferative DES agents sirolimus (CYPHER stent, Cordis, Miami Lakes, Florida), paclitaxel (Taxus stent, Boston Scientific, Natick, Massachusetts), zotarolimus (Endeavor stent, Medtronic, Minneapolis, Minnesota), and everolimus (Xience stent, Abbott and Boston Scientific) have potent antimitotic actions that strongly inhibit smooth muscle proliferation and matrix production (1-3) and thus reduce neointimal formation and restenosis. Despite efficacy in reducing neointimal proliferation and restenosis, DES failure and restenosis still occurs and is more frequent in the settings of diabetes mellitus and during treatment of restenotic lesions, bypass grafts, and bifurcations (4-6). In addition to restenosis, concern has arisen about the potential for late thromboses or very late thromboses after DES implantation, and this concern has led to extended-duration dual antiplatelet therapy (7-9). Mechanisms of stent thrombosis might vary, depending on the timing of the event (10). Acute stent thrombosis (within 24 h of implantation) and early stent thrombosis (within 30 days) are likely related to mechanical issues with the stent, inadequate platelet inhibition, or pro-thrombotic patient

risk factors. In contrast, late stent thrombosis (up to 1 year) and very late stent thrombosis (after 1 year) have been attributed to delayed re-endothelialization and inhibition of vascular repair. The potential for delayed re-endothelialization and inhibition of vascular repair is particularly important after implantation of DES, because the antiproliferative agents used to prevent smooth muscle cell proliferation also delay re-endothelialization in the stented segment (11,12). Angioscopic (13) and pathological (11,12,14,15) evidence suggests that there is delayed arterial healing with DES, compared with bare-metal stents (BMS), because DES-treated arteries have more histological evidence of incomplete re-endothelialization, chronic inflammatory cell infiltration, fibrin deposition, and platelet activation. It is important to recognize that inflammatory and thrombotic pathways share common signaling pathways and that inflammatory responses promote activation of the clotting cascade and stimulate platelet activation (reviewed in Croce and Libby [16]). Experimental studies also suggest that delayed arterial healing and DES-associated inflammation is greatest at sites of overlapping DES with placement of multiple stents (17). The finding of increased inflammation in areas of stent overlap suggests a possible molecular mechanism to explain higher stent thrombosis rates that are associated with overlapping stents.

||Department of Cardiovascular and Renal Medicine, Saga University Faculty of Medicine, Saga, Japan. This work was supported in part by grants from the National Heart, Lung, and Blood Institute to Dr. Simon (HL85816, HL57506 MERIT Award, HL73852) and to Dr. Croce (1K08HL086672); a Future Leaders in Cardiovascular Medicine Fellowship Grant to Dr. Croce; an award from the Michael Lerner Foundation to Dr. Croce; a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by a grant from Kimura Foundation to Drs. Inoue and Node; and by a

research grant from the Japan Foundation of Cardiovascular Research to Drs. Inoue and Node. Dr. Simon is on the advisory board and is a consultant for Cordis/Johnson & Johnson and Medtronic Vascular, and is a consultant for Daiichi-Sankyo. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. The first two authors contributed equally to this work.

Manuscript received August 20, 2010; revised manuscript received February 22, 2011, accepted May 3, 2011.

In addition to antiproliferative drug-associated delayed healing with DES, stent-induced or polymer-induced inflammation has also been identified as a possible contributor to stent thrombosis, especially because late and very late stent thrombosis occurs long after antiproliferative drugs have been eluted from the polymer (18–20). Inflammatory responses to drug, stent, or polymer might result from nonspecific innate immune responses, which have a predominance of monocyte/macrophage infiltrates, or might be related to antigen-specific adaptive immune hypersensitivity responses typified by infiltration of eosinophils, B-cells, and T-cells (reviewed in Byrne et al. [21]). Several studies have also implicated DES-polymer-induced inflammation in the pathobiology of restenosis and stent thrombosis (18,19). Currently, the 4 stent platforms approved for use by the U.S. Food and Drug Administration use different nonerodible polymeric coatings for drug delivery, and experimental animal studies suggest that biological compatibility, immunogenicity, and thrombogenicity might vary among specific polymeric compounds (22). The next generations of DES represent an attempt to reduce the possibility of polymer-induced inflammation, delayed arterial healing, restenosis, and stent thrombosis through use of polymers that have better biocompatibility and/or are biodegradable.

Emerging evidence indicates that compared to BMS, DES impair endothelial function in arterial segments distal to the stented site (23,24). Even 6 months after implantation of DES, artery segments distal to the DES show abnormal vasoreactivity (25–27). DES-associated abnormalities in endothelial function could be related to delayed vascular repair and not the DES drug itself, because the kinetics of DES are such that the drugs are completely eluted within months after implantation (28–31). It is possible, however, that in certain circumstances drug accumulation in the arterial wall (32) and the lipophilic core of stented atheroma results in prolonged drug retention/release and ongoing vascular dysfunction. The mechanism of DES-associated endothelial dysfunction is not established, and recent studies have demonstrated that there is variability in the severity of DES-associated endothelial dysfunction among specific DES agents (33–35). It is unclear whether DES-associated vascular dysfunction influences clinical outcomes after DES implantation. One small study demonstrated impaired endothelial function in patients presenting with in-stent restenosis, compared with matched control subjects (36); however, this association will require validation in larger prospective investigations.

Because of the potential for delayed re-endothelialization and repair with DES, concern was raised about possible increased mortality and late stent thrombosis following DES implantation (reviewed in Garg and Mauri [7]). Because of the insufficient power of individual trials to assess the low-incidence events of late and very late stent thrombosis, multiple meta-analyses were performed to evaluate

the risk of stent thrombosis in patients treated with DES versus BMS (37–41). These meta-analyses and subsequent analyses of stent registry data (42–45) demonstrated nearly equivalent risk of stent thrombosis (approximately 0.5%) in patients treated with DES or BMS. A small increase in the risk of late and very late stent thrombosis on the order of 1% to 2% cannot be excluded, however, because available data have insufficient power to evaluate this very rare event.

Analyses of stent thrombosis and outcomes with DES are further complicated by significant differences in stent structure, drug delivery polymers, and antiproliferative drugs among the rapidly expanding panel of DES. In addition, complex biology controls vascular repair after percutaneous coronary intervention (PCI). Understanding the common and differential molecular pathways that regulate re-endothelialization versus restenosis will provide a biological context for rational use of DES and will enable development of new DES technologies that can inhibit neointimal proliferation and preserve or even promote endothelial repair. In the following sections, we will highlight key cellular and molecular pathways that regulate vascular injury and repair in the setting of percutaneous coronary revascularization, and we will discuss the role of DES in modulating vascular repair processes.

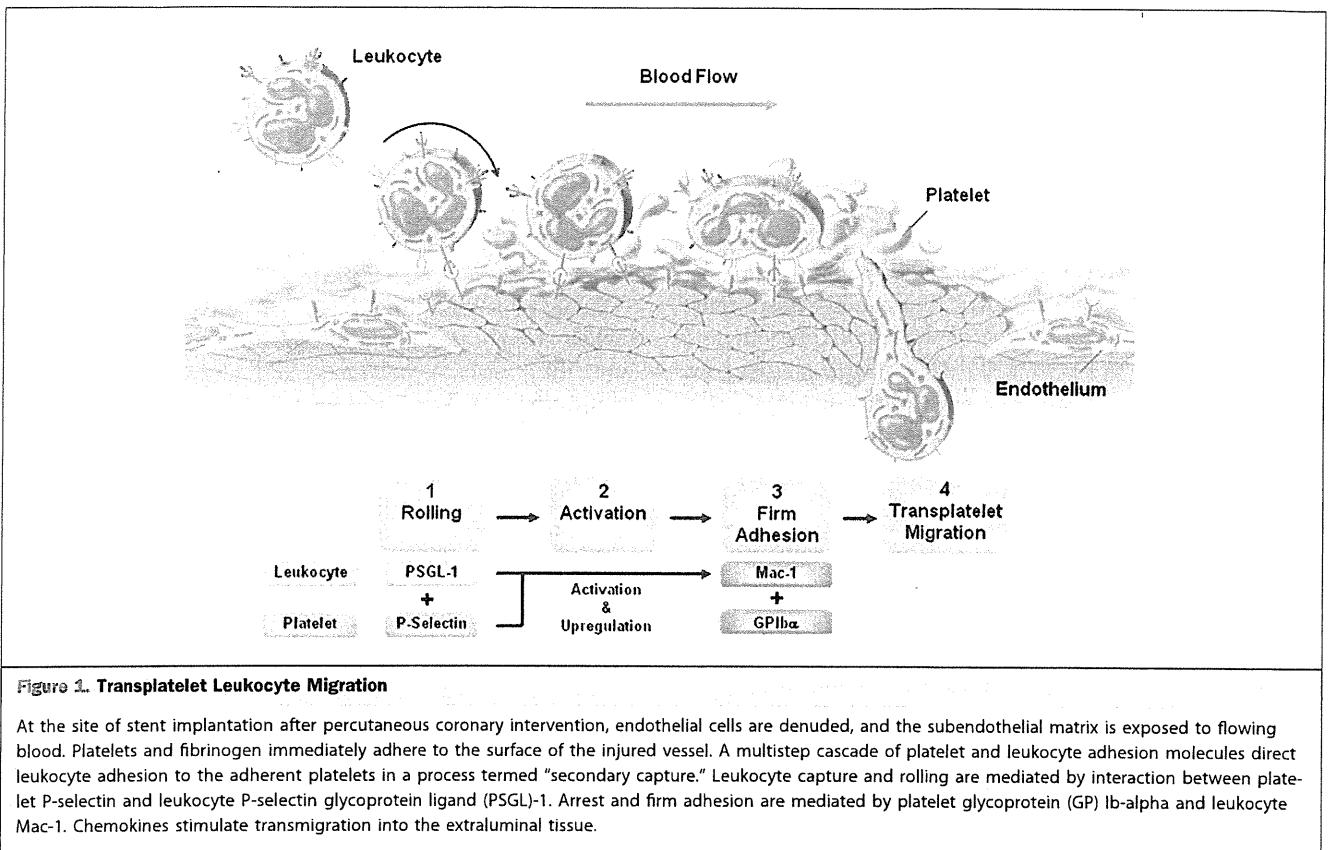
Role of Inflammation in Restenosis and Vascular Repair

Stent placement leads to mechanical injury that induces substantial local inflammation, which stimulates vascular smooth muscle cell proliferation and extracellular matrix deposition, resulting in neointimal thickening and restenosis (46,47). Vascular inflammation after PCI involves complex interactions between multiple vascular cell types, and under normal circumstances, the cellular and molecular processes that control vascular injury responses direct repair and vascular healing. In pathological conditions, dysregulation of vascular repair results in persistent vascular inflammation, neointimal proliferation, and restenotic obstruction of the stent lumen.

Immediately after PCI, platelets, neutrophils, and monocytes play a central role in the initial inflammatory response (47,48). Platelets and fibrin deposit on the de-endothelialized vessel wall and recruit leukocytes to the injured vessel segment through a cascade of cell adhesion molecules that direct leukocyte attachment and transmigration across surface-adherent platelets (49). The initial tethering and

Abbreviations and Acronyms

BMS = bare-metal stent(s)
DES = drug-eluting stent(s)
EPC = endothelial progenitor cell
G-CSF = granulocyte colony-stimulating factor
PCI = percutaneous coronary intervention
SDF = stromal cell-derived factor
SMPC = smooth muscle progenitor cell



rolling of leukocytes on platelets is mediated through binding of the leukocyte receptor P-selectin glycoprotein ligand-1 to platelet P-selectin (50–52). Rolling leukocytes stop and firmly attach to adherent platelets when the leukocyte integrin Mac-1 (CD11b/CD18) binds to platelet glycoprotein Ib-alpha (53) or to fibrinogen bound to the platelet glycoprotein IIb/IIIa (Fig. 1) (54). A direct role for Mac-1 in leukocyte adhesion after mechanical injury has been demonstrated in several experimental studies where Mac-1 targeting reduces neointimal thickening after experimental angioplasty (55,56). Clinical studies of patients undergoing PCI further support the premise that Mac-1 and platelet-mediated leukocyte adhesion (also termed "secondary capture") plays an important role in vascular inflammation and restenosis after coronary stenting. We have previously shown that, compared with circulating neutrophils, Mac-1 surface expression is significantly increased in the neutrophils obtained from the coronary sinus of patients who underwent PCI within the preceding 48 h and that high levels of Mac-1 expression are associated with angiographic late lumen loss and increased risk of restenosis (57–60). Increased Mac-1 expression also correlates with increased expression of P-selectin on the surface of platelets obtained from the coronary sinus after PCI (57–60).

Role of Bone Marrow-Derived Stem Cells in Restenosis and Vascular Repair

Emerging research is demonstrating that bone marrow-derived progenitor cells play an important role in vascular inflammation responses and in vascular repair. Endothelial progenitor cells (EPCs) mobilized from bone marrow into peripheral blood promote endothelial regeneration and postnatal neovascularization (61,62). In contrast to the potential protective effects of EPCs, it has been hypothesized that smooth muscle progenitor cells (SMPCs), which are also mobilized from bone marrow, migrate to the sites of vascular injury where they contribute to smooth muscle cell expansion and neointimal proliferation (63–65).

The precise function of EPCs and SMPCs once they home to sites of vascular inflammation is controversial. Previously, CD34-positive cells were believed to be committed to develop into EPCs; however, further study demonstrated that the CD34 surface antigen actually identifies undifferentiated bone marrow-derived stem cells that have the ability to differentiate into EPC and SMPCs. Transdifferentiation of CD34-positive cells into EPC or SMPC lineages depends on the local environment; ischemic conditions signal differentiation toward EPC phenotypes to promote re-endothelialization (61,66), and inflammatory

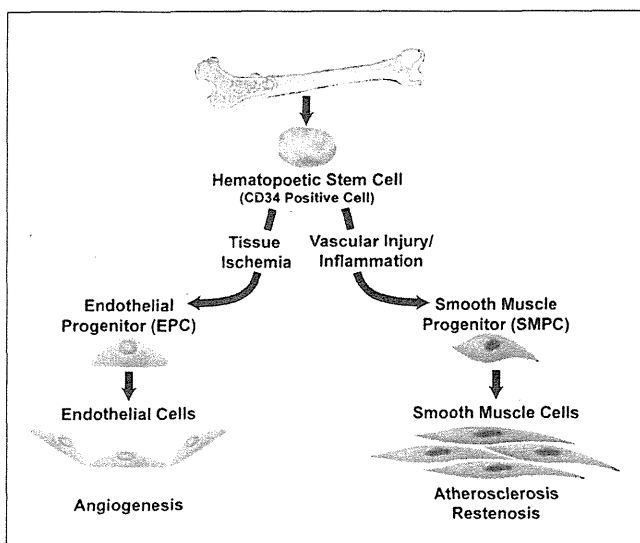


Figure 2. Differentiation of Bone Marrow-Derived Stem Cells

Previously, CD34-positive cells were believed to be committed to develop into EPCs; however, further study demonstrated that the CD34 surface antigen actually identifies undifferentiated bone marrow-derived stem cells that have the ability to differentiate into EPC and SMPCs. Ischemic conditions signal differentiation toward EPC phenotypes to promote re-endothelialization. Inflammatory conditions signal differentiation toward SMPC phenotypes that promote neointimal proliferation.

conditions signal differentiation toward SMPC phenotypes that promote neointimal proliferation (63) (Fig. 2).

Several studies have implicated CD34-positive progenitor cells in vascular injury responses after PCI. Circulating CD34-positive cells are increased in the days after acute myocardial infarction, and characterization of these circulating cells suggests that they have an EPC-like phenotype, raising the possibility that CD34-positive EPC-like cells are mobilized to promote angiogenesis in the ischemic myocardium. In contrast to ischemia-mediated mobilization, SMPC-like CD34-positive cells increase after PCI in patients with chronic coronary artery disease, presumably in response to inflammatory mediators produced at sites of stent implantation (67). In this setting, elevated levels of circulating CD34-positive cells are associated with increased rates of restenosis, suggesting possible involvement in neointimal formation (68).

We have also demonstrated that molecular signals generated at sites of local arterial inflammation promote the mobilization of CD34-positive stem cells (69). In our study, the number of CD34-positive cells in the peripheral blood increased Day 7 to 14 after PCI, and patients who received BMS had significantly more CD34-positive cells than those who received DES (Fig. 3A) (69,70). Granulocyte colony-stimulating factor (G-CSF) and Mac-1 levels were significantly reduced in patients who underwent implantation of DES, compared with those who received BMS, suggesting that the antiproliferative stent drug attenuated inflamma-

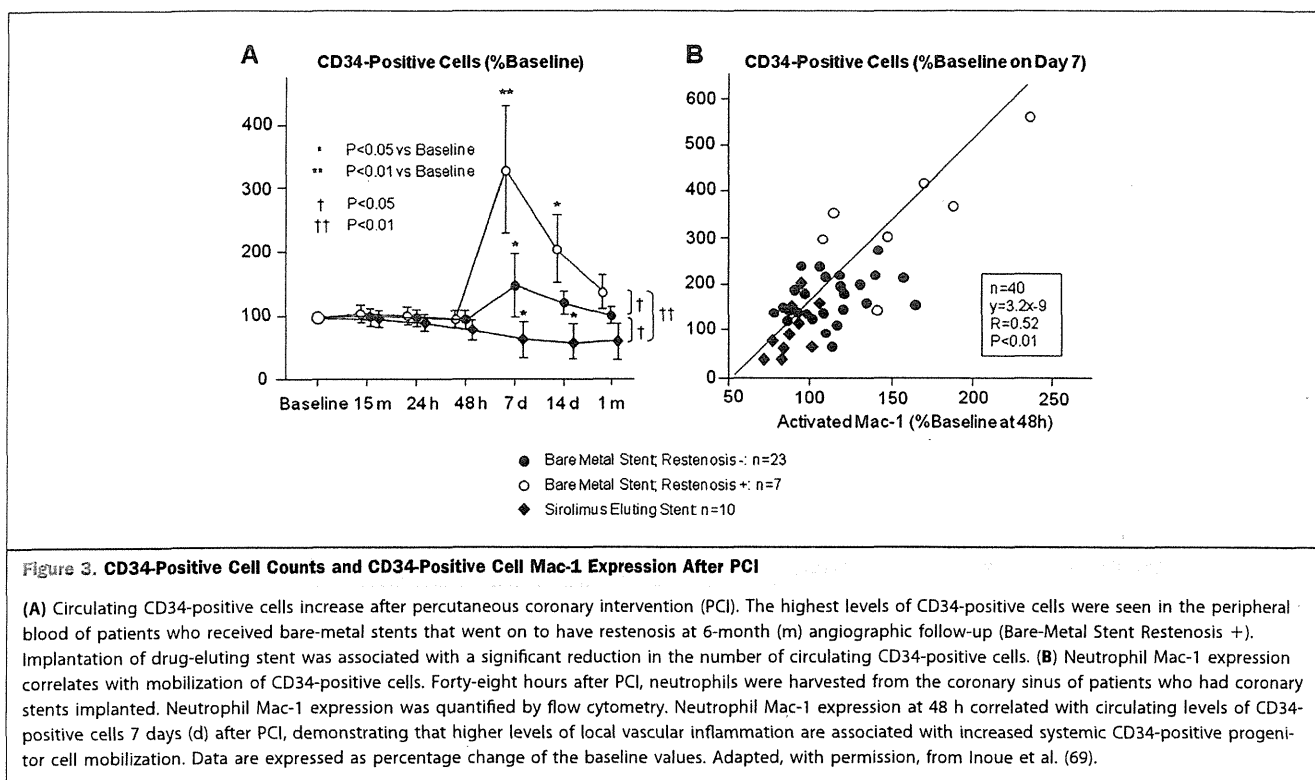
tory cell activation (Fig. 3B) (69). This observation is consistent with our hypothesis that inflammatory signals generated at sites of coronary injury mobilize bone marrow-derived progenitor cells involved in vascular repair. To further elucidate the role of CD34-positive cells in vascular injury and repair after PCI, we isolated circulating CD34-positive progenitor cells from patients who received DES and BMS and performed *in vitro* differentiation assays (Fig. 4) (69). In most patients, a proportion of the cultured CD34-positive cells differentiated into both CD31-positive endothelial-like cells and into alpha-actin-positive cells with features suggestive of smooth muscle cell lineage. Several other observations were made. First, the number of differentiated colonies that formed from the CD34-positive cells correlated with the extent of restenosis during angiographic follow-up. Second, patients with more angiographic restenosis had more CD34-positive cells that differentiated into alpha-actin containing SMPC-like cells. Third, implantation of sirolimus-eluting stents resulted in reduced differentiation of CD34-positive cells into CD31-positive cells and reduced differentiation into alpha-actin-positive cells with smooth muscle cell features. This finding is consistent with *in vitro* data demonstrating that sirolimus inhibits differentiation of human bone marrow-derived stem cells into endothelial or smooth muscle cells (71,72).

Several lines of evidence support the premise that PCI induces local inflammatory signals that mobilize bone marrow-derived CD34-positive stem cells and that these cells have the ability to differentiate along endothelial or smooth muscle cell lines. In the setting of vascular injury, there seems to be a balance between endothelial-like stem cell responses that favor re-endothelialization and smooth muscle-like stem cell responses that promote restenosis (Fig. 2). Furthermore, it seems that, compared with BMS, sirolimus-eluting stent implantation attenuates production of local inflammatory signals that promote stem cell mobilization and differentiation into smooth muscle-like cells that contribute to neointimal proliferation. In the future, targeted pharmacological therapies might be able to promote reparative progenitor cell responses and/or inhibit responses that result in excess neointimal proliferation.

Local Vascular Inflammation Signals Stem Cell Recruitment

As described in the preceding text, inflammatory and hematopoietic cytokines produced locally at sites of vascular inflammation direct mobilization of stem cells from the bone marrow. Vascular-derived molecules involved in stem cell mobilization include G-CSF, matrix metalloproteinase-9, and stromal cell-derived factor (SDF)-1.

G-CSF, a potent hematopoietic cytokine produced by endothelium and immune cells, is expressed at sites of



vascular injury (73). G-CSF promotes stem cell proliferation and mobilization, and it has been hypothesized that, after PCI and/or myocardial infarction, G-CSF signals production and homing of reparative stem cells that promote angiogenesis and myocardial repair. Clinical evaluation of systemic G-CSF therapy after myocardial infarction failed to show benefit in limiting infarct size or in improving left ventricular function, despite its experimental effects on stem mobilization (74,75). It is possible that the nonselective mobilization of both EPCs and SMPCs by G-CSF might limit its therapeutic value for treating restenosis and promoting vascular repair.

Neutrophil-derived matrix metalloproteinase-9 is another inflammatory mediator that has a role in stem cell mobilization (76). Matrix metalloproteinase-9 is secreted locally in response to inflammatory inputs, including ligand binding to the leukocyte integrin Mac-1 (77). Matrix metalloproteinase-9 is required for G-CSF and chemokine-induced mobilization of hematopoietic stem cells from the bone marrow (78,79) and provides a mechanism through which inflamed vascular beds generate systemic signals that promote bone marrow-derived stem cell mobilization.

SDF-1 is a member of the CXC group of chemokines that plays a role in stem cell plasticity and engraftment (80). SDF-1 is expressed by smooth muscle cells at sites of atherosclerosis and vascular inflammation. SDF-1 signals the bone marrow to mobilize Sca-1⁺ lineage progenitor

cells that home to sites of vascular injury where the progenitor cells adopt smooth muscle cell phenotypes. In experimental models, SDF-1 directly regulates neointimal smooth muscle cell content, and inhibition of SDF-1 function decreases neointimal formation (80). Therapies targeting SDF-1 function could potentially inhibit restenosis after PCI.

Modulating Vascular Injury and Repair: New Frontiers in DES Technology

Current-generation DES agents prevent restenosis by inhibiting smooth muscle cell proliferation. In developing the next generation of DES agents it might be possible to harness differential drug effects on smooth muscle cell proliferation versus re-endothelialization in a manner that could accelerate repair. Vascular endothelial growth factor has attracted attention as a DES agent that could promote endothelial regeneration and angiogenesis (81). Proof-of-concept investigations have demonstrated that vascular endothelial growth factor gene-eluting stents accelerate re-endothelialization and reduce in-stent neointimal area in animal models (82). Another new strategy to promote vascular repair after PCI involves the use of antibodies (83) or peptides (84) that bind membrane receptors on circulating endothelial progenitor cells. This strategy promotes capture of these cells to accelerate healing (83). CD34 antibody-coated stents have been implanted in human

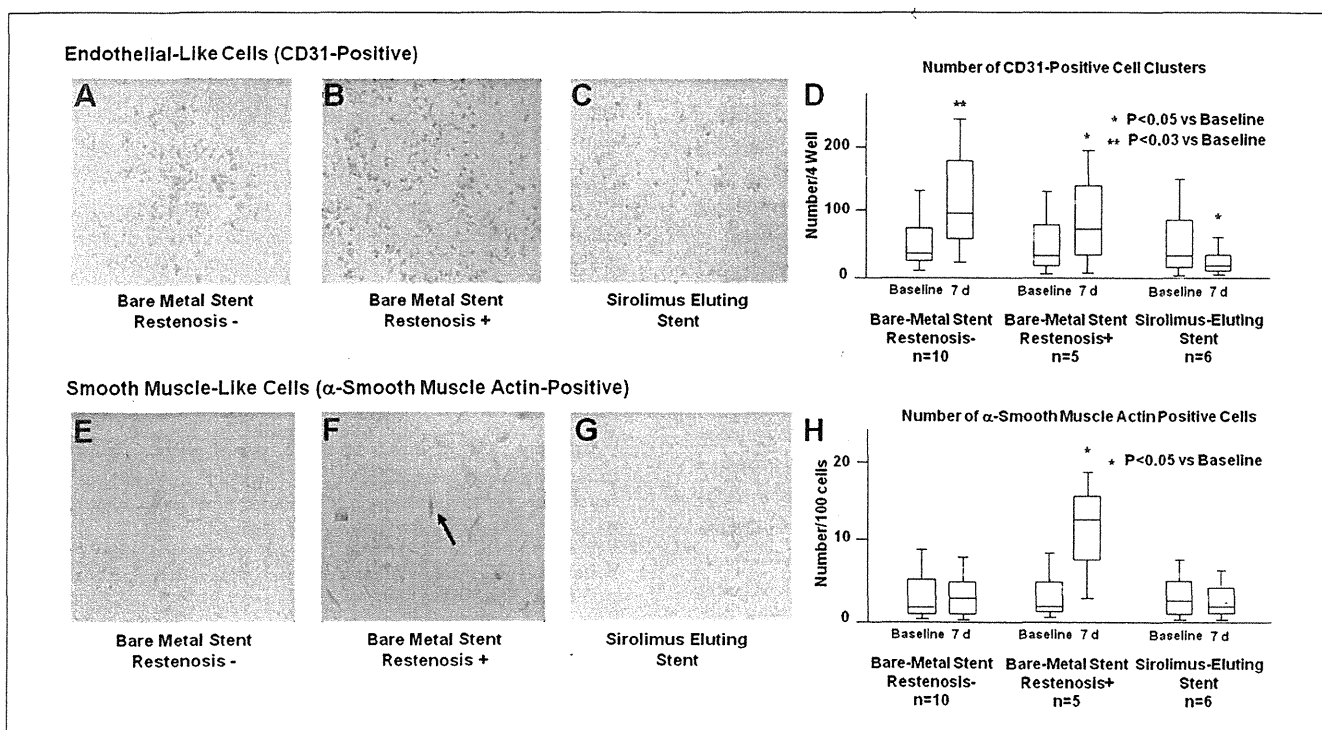


Figure 4. Differentiation of Patient-Derived CD34-Positive Stem Cells Into Endothelial-Like and Smooth Muscle-Like Cells After PCI

Circulating CD34-positive stem cells were isolated from peripheral blood of patients 7 days (d) after implantation of bare-metal stent (BMS) or sirolimus-eluting stent (SES). Immunohistochemical staining for CD31 (**A to D**). (**A**) BMS without restenosis, (**B**) BMS with restenosis, (**C**) SES, (**D**) quantification of CD31-positive cell clusters. Patients who received BMS had similar differentiation of CD34-positive stem cells into CD31-positive endothelial-like cells, regardless of whether they went on to have restenosis at 6-month angiographic follow-up. Patients who received SES had a significant reduction in the differentiation of CD34-positive stem cells into CD31-positive endothelial-like cells, compared with patients that received BMS. Actin staining (**E to H**). (**E**) BMS without restenosis, (**F**) BMS with restenosis, (**G**) SES, (**H**) quantification of actin positive cells. Patients who received BMS and went on to have restenosis at 6-month angiographic follow-up had increased numbers of CD34-positive stem cells that differentiated into actin-positive smooth muscle-like cells. Patients who received SES had a significant reduction in the differentiation of CD34-positive stem cells into actin-positive smooth muscle-like cells, compared with patients that received BMS. **Arrow** denotes representative actin-positive cell. Adapted, with permission, from Inoue et al. (69).

coronary arteries in the multicenter HEALING (Healthy Endothelial Accelerated Lining Inhibits neointimal Growth) II pilot trial and in later follow-up studies (85,86). The long-term safety and efficacy of this pro-healing stent technology awaits further evaluation in randomized trials.

In addition to DES technology itself, adjunctive systemic medications might also influence stem cell homing and the balance between re-endothelialization and neointimal proliferation. Interestingly, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) were recently shown to promote EPC proliferation in vitro (87) and increase the number of circulating EPCs in patients with coronary artery disease (88). Despite initial optimism that statins might favorably influence arterial healing after DES implantation, enthusiasm has tempered after release of data showing that high doses of statins started before PCI and continued thereafter increased EPC mobilization but did not increase circulating CD34⁺ cells and did not improve the angiographic outcome after implantation of a bioengineered EPC-capture stent (89):

Thiazolidinediones, which are used to treat diabetes, function by activating peroxisome proliferator activating receptor transcription factors. Several thiazolidinedione agents increase the number of EPCs in both circulating blood and bone marrow and reduce EPC apoptosis in a phosphatidylinositol 3-kinase-dependent manner (90). Although there are several potential vasculoprotective actions of statins and thiazolidinediones, further clinical investigation will be required to determine whether these medications will positively influence vascular repair, resulting in reduced rates of restenosis and enhanced re-endothelialization after PCI.

Conclusions

Percutaneous coronary intervention results in mechanical injury that induces vascular inflammation. Vascular inflammation involves complex interactions between endothelial cells, smooth muscle cells, platelets, and inflammatory cells, including neutrophils, monocytes, and lymphocytes. Signaling molecules produced by cells at the site of vascular injury

stimulate mobilization of bone marrow-derived EPCs and SMPCs, which are recruited to the sites of vascular inflammation. The cellular and molecular processes that control vascular injury responses direct repair and vascular healing; however, dysregulation of these responses can result in adverse arterial remodeling, neointimal proliferation, and restenosis. Drug-eluting stents effectively reduce neointimal proliferation but they slow re-endothelialization and healing. Drug-eluting stents also seem to influence the mobilization, homing, and differentiation of reparative stem cells. Despite the potential for DES-induced delayed vascular healing, clinical trial investigations have demonstrated similar safety of DES and BMS in the setting of extended dual antiplatelet therapy. In the future, improved DES technologies have the potential to abolish restenosis and further improve stent safety by inhibiting maladaptive neointimal proliferation while promoting re-endothelialization and repair.

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Key Words: inflammation ■ re-endothelialization ■ restenosis ■ stent thrombosis.

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