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Low testosterone level as a predictor of cardiovascular events in Japanese men with coronary risk factors

Masahiro Akishita[a,*], Masayoshi Hashimoto[b], Yumiko Ohike[a], Sumito Ogawa[a], Katsuya Iijima[a], Masato Eto[a], Yasuyoshi Ouchi[a]

[a] Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
[b] Department of General Internal Medicine, Kobe University School of Medicine, Kobe, Japan

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ABSTRACT

Objective: Recent epidemiological studies have found that testosterone deficiency is associated with higher mortality largely due to cardiovascular (CV) disease in community-dwelling older men. We investigated whether a low plasma testosterone level could predict cardiovascular events in middle-aged Japanese men with coronary risk factors.

Methods: One hundred and seventy-one male outpatients (30–69 years old, mean ± SD = 48 ± 13 years) who had any coronary risk factor (hypertension, diabetes, dyslipidemia, smoking, and obesity) without a previous history of CV disease were followed up. At baseline, the subjects underwent examination of coronary risk factors, measurement of flow-mediated dilation (FMD) of the brachial artery as an indicator of vascular endothelial function and assays of plasma total testosterone, dehydroepiandrosterone-sulfate (DHEA-S), estradiol and cortisol.

Results: During the mean follow-up period of 77 months, a total of 20 CV events occurred. Kaplan–Meier survival analysis by tertile of plasma hormone levels revealed that the subjects with the lowest testosterone tertile were more likely to develop CV events than those with the highest tertile ($P < 0.01$ by log-rank test). Cox proportional hazards models showed that the subjects with the lowest tertile of plasma testosterone (<14.2 nmol/L) had an approximately 4-fold higher CV event risk compared to those with the higher testosterone tertiles after adjustment for coronary risk factors including medication and FMD (unadjusted hazard ratio, 3.61; 95% CI, 1.47–8.86: multivariate-adjusted hazard ratio, 4.61; 95% CI, 1.02–21.04). Multivariate analysis did not show any significant association of DHEA-S, estradiol or cortisol with CV events.

Conclusions: A low plasma testosterone level is associated with CV events in middle-aged Japanese men, independent of coronary risk factors and endothelial function. This is the first report to show the relationship between endogenous testosterone and CV events in Asian population.

1. Introduction

Plasma testosterone level declines with advancing age in men [1]. Testosterone deficiency is often associated with age-related diseases such as erectile dysfunction, osteoporosis, depressed mood, cognitive impairment and frailty [2,3]. Furthermore, a number of studies suggest that testosterone deficiency is related to cardiovascular (CV) disease and its risk factors in men. Inverse relations between testosterone level and coronary risk factors including obesity [4,5], hypertension [5,6], dyslipidemia [4,5], and diabetes [5,7] have been reported. In addition, we and others have shown that a low testosterone level is associated with markers of atherosclerosis such as impaired endothelial vasomotor function [8], increased carotid intima-media thickness [9] and aortic calcification [4]. Although these data do not indicate a causal relationship between endogenous testosterone and CV disease, recent epidemiological studies have demonstrated that community-dwelling older men with a low testosterone level are more likely to die [10–12], largely due to CV disease [11,12]. However, this issue remains unknown in Asian population.

Based on these backgrounds, we tested the hypothesis that a low testosterone level is an independent risk factor for CV disease even in middle-aged Japanese men with coronary risk factors. For this purpose, we conducted a survey of 171 male patients by using baseline clinical information and by measuring sex hormone levels in stored plasma.

* Corresponding author. Tel.: +81 3 5800 8832; fax: +81 3 5800 8831.
E-mail address: akishita-tky@umin.ac.jp (M. Akishita).

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2. Methods

2.1. Subjects

Male subjects aged 30–69 years at baseline, who were referred to our department to check for CV disease and undergo examination of vasomotor function of the brachial artery in 1996–2000, and had any of the classical coronary risk factors including hypertension, dyslipidemia, diabetes mellitus and current smoking, were eligible. Hypertension, dyslipidemia and diabetes mellitus were defined according to diagnostic criteria [13–15] or if the subject was taking any medication for these diseases. Subjects with a history of CV disease, including stroke, coronary heart disease, congestive heart failure and peripheral arterial disease, were excluded. Malignancy, overt endocrine disease and use of steroid hormones were also excluded, because these conditions may have a significant influence on both plasma sex hormones and clinical course.

Of the 188 eligible subjects whose plasma was stored, written informed consent was obtained from 171 subjects; 1 subject refused and 16 subjects were lost to follow-up. Then, plasma hormone levels were measured and follow-up data were obtained in 171 subjects. The study protocol was approved by the ethics committee of the Graduate School of Medicine, The University of Tokyo. Each subject or a family member, if the subject had died, gave written informed consent for enrollment in this study.

2.2. Clinical measurements

Clinical information was collected at baseline when each patient attended our department. Blood sampling and measurement of height, weight, blood pressure and vasomotor function were performed in the morning after a 14-h overnight fast. Blood pressure was measured at least twice using an automated, digital electrosphygmomanometer (Omron Healthcare Co., Ltd., Kyoto, Japan) on the nondominant arm in a sitting position, and the average was used for analysis.

Serum total cholesterol and triglyceride concentrations were measured enzymatically, and serum high-density lipoprotein (HDL) cholesterol concentration was measured by the heparin-$Ca^{2+}Ni^{2+}$ precipitation method. Plasma glucose concentration was assayed by the glucose oxidase method, and hemoglobin A1c level was measured by high-performance liquid chromatography.

Plasma concentrations of total testosterone, dehydroepiandrosterone-sulfate (DHEA-S), estradiol and cortisol were determined using sensitive radioimmunoassays by a commercial laboratory (SRL, Inc., Tokyo, Japan). Because the plasma used for hormone assays was deep-frozen (−80 °C) for up to 7 years, we checked the change in titers using the stored samples, which had been measured at sampling 5–7 years before. Pearson's correlation coefficient between the two measurements was 0.965 for estradiol (n = 34), 0.976 for testosterone (n = 20), 0.991 for DHEA-S (n = 15) and 0.937 for cortisol (n = 16), indicating that there was no significant change in plasma titers in our frozen samples. The intra-assay coefficients of variation for the measurements were less than 5%.

Vasomotor function of the brachial artery was evaluated using an ultrasound machine according to the method described previously [16]. Briefly, endothelium-dependent flow-mediated vasodilation (%FMD) was measured as the maximal percent change in the vessel diameter after reactive hyperemia. Subsequently, endothelium-independent nitroglycerin-induced vasodilation was measured as the maximal percent change in the vessel diameter after sublingual administration of nitroglycerin spray (0.3 mg; Toa Eiyo Co., Tokyo). The same examiner (M.H.) performed the measurements of FMD throughout this study.

2.3. Follow-up

The subjects were followed in 2006–2007 by mail and/or visits to our clinic. Each subject or a family member completed the questionnaire on CV disease and health status. CV events analyzed as the endpoints of this study included stroke, coronary artery disease, sudden cardiac death, and peripheral arterial disease. If CV events were reported on the questionnaire, we attempted to confirm the diagnosis of each event by medical records and/or interview by research doctors who were unaware of the patient's plasma hormone levels. Finally, after thorough examination, 20 cases were determined as CV events. Eighteen cases were ascertained by medical records which included clinical course, physical examination, laboratory tests and imagings. Because medical records were not available on other two cases of self-reported ischemic stroke, they were diagnosed according to the phone interview to each patient.

2.4. Data analysis

Values are expressed as mean ± SD in the text unless otherwise stated. Differences between the groups were analyzed using ANOVA for continuous variables and Chi-squared test for categorical variables. Survival was analyzed using Kaplan–Meier plots and log-rank tests. Hazard ratios (HRs) for CV events were analyzed using Cox proportional hazards regression. A value of $P < 0.05$ was considered statistically significant. Data were analyzed using SPSS (Ver. 17.0, SPSS Inc., Chicago, IL).

3. Results

3.1. Characteristics of subjects according to plasma testosterone level

Table 1 shows the baseline characteristics of the subjects by tertile of plasma testosterone. As reported previously [4–8], subjects with the lowest testosterone tertile tended to be obese, hypertensive, dyslipidemic, diabetic, and to have impaired endothelial vasomotor function compared to those with higher testosterone tertiles. Age and smoking status were not different between the groups.

3.2. CV events and hormones

During the mean follow-up period of 77 ± 46 months (median = 54 months), a total of 20 CV events occurred (Table 2). Eleven cases of coronary artery disease included three of myocardial infarction, three of medically treated angina pectoris, four of percutaneous coronary intervention, and one of coronary artery bypass grafting. All of the five cases of stroke were due to cerebral infarction.

As shown in Fig. 1, Kaplan–Meier survival analysis by tertile of plasma testosterone level revealed that low testosterone was associated with CV events. Cox proportional hazards models showed that the subjects with the lowest tertile of plasma testosterone, but not those with the middle tertile, had significantly increased risk for CV events compared to those with the highest tertile (Table 2). Adjustment for age and body mass index did not attenuate the effect.

Then, HRs for the lowest tertile of plasma testosterone vs. the higher (middle and highest) tertiles were analyzed. The subjects with the lowest tertile (<14.2 nmol/L) showed an unadjusted HR of 3.61 (95% CI, 1.47–8.86), and an adjusted HR of 4.24 (95% CI, 1.67–10.78) for age, body mass index, and current smoking. The HR was 4.61 (95% CI, 1.02–21.04) after adjustment for age, body mass index, current smoking, systolic blood pressure, HDL cholesterol, non-HDL cholesterol, hemoglobin A1c, %FMD,

Table 1
Baseline characteristics of subjects by tertile group of plasma testosterone.

	Tertile 1 <14.2 nmol/L (n=57)	Tertile 2 14.2–19.4 nmol/L (n=57)	Tertile 3 >19.4 nmol/L (n=57)	p for trend
Testosterone (nmol/L)	11.0±3.0	17.0±1.6	24.0±3.0	<0.001
(ng/dL)	(318±86)	(490±45)	(693±86)	
DHEA-S (μmol/L)	4.94±2.68	4.55±2.25	4.83±2.64	0.81
Estradiol (pmol/L)	115±30	116±31	133±30	0.004
Cortisol (nmol/L)	386±138	378±142	361±120	0.67
Age (years)	47±13	45±13	50±14	0.24
Body mass index (kg/m^2)	27.6±5.5	25.6±4.3	24.1±3.6	<0.001
Systolic blood pressure (mmHg)	131±18	125±16	123±12	0.01
Diastolic blood pressure (mmHg)	79±15	74±11	74±9	0.04
Non-HDL cholesterol (mmol/L)	4.19±1.27	3.91±1.06	3.74±1.01	0.10
HDL cholesterol (mmol/L)	1.20±0.36	1.23±0.41	1.44±0.48	0.005
Triglycerides (mmol/L)	2.04±2.12	1.91±1.85	1.46±1.28	0.18
Fasting plasma glucose (mmol/L)	6.00±1.18	5.73±0.92	5.73±1.28	0.34
Hemoglobin A1c (%)	5.9±1.7	5.2±0.8	5.5±1.2	0.03
%FMD	4.2±2.7	5.7±4.2	6.1±3.8	0.01
%NTG	12.8±4.3	14.2±5.4	13.2±5.0	0.30
Hypertension, n (%)	30 (53)	20 (35)	20 (35)	0.09
Dyslipidemia, n (%)	33 (58)	35 (61)	24 (42)	0.09
Diabetes mellitus, n (%)	15 (26)	7 (12)	9 (16)	0.13
Current smoker, n (%)	28 (49)	25 (44)	29 (51)	0.74

DHEA-S, dehydroepiandrosterone-sulfate; HDL, high-density lipoprotein; %FMD, percent flow-mediated dilation of brachial artery; %NTG, percent nitroglycerine-induced dilation of brachial artery.
Values are expressed as mean ± SD. Continuous variables were compared by ANOVA and categorical variables by Chi-squared test.

Table 2
Cardiovascular events by tertile of plasma testosterone.

	Tertile 1 <14.2 nmol/L (n=57)	Tertile 2 14.2–19.4 nmol/L (n=57)	Tertile 3 >19.4 nmol/L (n=57)	Total (n=57)
Number of events				
Stroke	2	3	0	5
Coronary artery disease	7	2	2	11
Sudden cardiac death	2	0	0	2
Peripheral arterial disease	1	0	1	2
Total cardiovascular events	12	5	3	20
HRs (95% CI) for total cardiovascular events				
Unadjusted	4.82 (1.36, 17.12)	1.67 (0.40, 6.99)	1(Ref)	
Adjusted for age	6.36 (1.78, 22.80)	1.82 (0.43, 7.71)	1(Ref)	
Adjusted for age and BMI	7.01 (1.94, 25.34)	1.86 (0.44, 7.86)	1(Ref)	

BMI, body mass index. HRs (Hazard ratios) were analyzed using Cox proportional hazards regression.

medications (antihypertensives, statins, hypoglycemic agents and antiplatelet agents), estradiol and DHEA-S. In addition to testosterone, age (HR per year, 1.12; 95% CI, 1.05–1.20), %FMD (HR per 1% increase, 0.80; 95% CI, 0.64–0.99) and HDL cholesterol (HR per 1 mg/dL, 0.88; 95% CI, 0.81–0.95) were independently associated with CV events, but other variables were not in this final model. Further inclusion of other hormones and nitroglycerin-induced endothelium-independent vasodilation into the model did not influence the statistical results (data not shown).

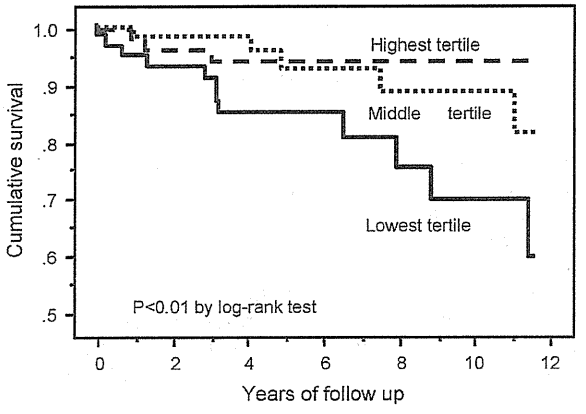

Fig. 1. Survival curves for cardiovascular events by tertile group of plasma concentration of testosterone. Cut-offs of the tertiles for testosterone were 14.2 and 19.4 nmol/L (410 and 560 ng/dL).

Two subjects with the lowest tertile of plasma testosterone suffered CV events within 6 months of follow-up; a case of sudden cardiac death and a case of coronary artery bypass grafting. Accordingly, similar statistical analyses were performed excluding these two cases. The results were essentially unchanged, although the HRs were slightly smaller (unadjusted HR, 3.06; 95% CI, 1.21–7.78: multivariate-adjusted HR, 3.80; 95% CI, 1.06–13.52).

Among other hormones examined, only DHEA-S was associated with increased risk for CV events, but was canceled by adjustment for age (data not shown). Further multivariate analysis did not show any significant association of DHEA-S, estradiol or cortisol with CV events.

4. Discussion

In this follow-up study of middle-aged Japanese men with coronary risk factors, a low plasma testosterone level was associated with CV events. Although the subjects with lower testosterone levels had worse profiles of coronary risk factors [4–7,11,12] and endothelial function [8] at baseline, as reported previously, adjustment for these confounders including age and cardiovascu-

lar medication indicated that low testosterone was an independent risk factor for CV events. In contrast, DHEA-S, estradiol and cortisol levels were not related to CV events.

A number of cross-sectional studies have shown an association between low testosterone level and CV disease [17,18], but have not provided evidence of a causal relationship between them. In recent years, longitudinal follow-up studies have demonstrated that community-dwelling older men (around 70 years on average) with lower testosterone levels are more likely to die from CV disease [11,12]. In contrast, a low testosterone level was not associated with CV deaths [19] or events [20] in community-dwelling middle-aged men (early 50s on average). These different findings might arise from the characteristics of the populations such as age and coronary risk factors, duration of follow-up and/or cut-off level of plasma testosterone at baseline. In any case, since all the above-mentioned studies were achieved in Caucasians, our study is the first to investigate the relationship between endogenous testosterone and CV events in Asians. Also, the present study showed a positive association between low testosterone level and CV events in middle-aged men with coronary risk factors, implying the clinical importance of measuring plasma testosterone in patients at risk, even if they are not old.

Unlike the previous reports showing an association of CV events with low levels of DHEA-S [21] and estradiol [22], and with a high cortisol:testosterone ratio [20], the present study did not show any significant association of CV events with estradiol, cortisol or cortisol:testosterone ratio (data not shown). The association between low DHEA-S and CV events was abolished by statistical adjustment for age, suggesting that the age-dependent decline of DHEA-S (Pearson's correlation coefficient between age and DHEA-S: −0.588; $P<0.001$) might have eliminated the association with CV events if present. Taking together with the Cox regression model including all hormones, it is suggested that testosterone is the strongest among four steroid hormones that could be predictive of CV events in this population.

There could be several mechanisms by which endogenous testosterone protects men from CV disease. Consistent with the present study, observational studies [4–8,11,12] suggest that testosterone might prevent risk factors such as obesity, hypertension, dyslipidemia, diabetes and endothelial dysfunction. Supplementary studies support the beneficial effects of testosterone on adiposity [23] and endothelial vasomotor function [24]. Based on these findings, risk markers and endothelial vasomotor function were entered into the multivariate models. Although statistical adjustment may have been insufficient to exclude the interaction between testosterone and these risk factors, testosterone remained a significant predictor of CV events in the present study. Testosterone has been reported to inhibit vascular smooth muscle cell proliferation and neointima formation [25], suggesting the direct action of testosterone on the vasculature. Also, the effects of testosterone on inflammation, hemostasis and cardiac ischemia [26] might be involved in the final process leading to CV events. The precise mechanisms, including the role of the androgen receptor and aromatization to estrogen, should be addressed in the future.

The finding of this study should not be extended to men without coronary risk factors. Our preliminary data of 47 middle-aged men without coronary risk factors showed that no subject suffered CV events during the mean follow-up period of 102 months, although a quarter of them had plasma testosterone level below the cut-off of this study (<14.2 nmol/L). Thus, the relationship between plasma testosterone and CV outcomes might be totally different in middle-aged Japanese men without coronary risk factors.

This study has several limitations. First, the number of CV events was too small to reach a clear conclusion with strong statistical power, due primarily to the small sample size and secondarily to the low incidence of CV events (approximately 2%/year). Second, the largely retrospective design (the protocol had been approved a few years before the final data collection) reduced the quality of the study and compelled us to lose many plasma samples and 16 subjects in the follow-up. Third, not all the CV events were confirmed by medical recordings. Two cases (a case in the lowest tertile and another in the middle tertile of plasma testosterone level) were determined according to the phone interview to each patient. Although the exclusion of these two cases did not significantly influence the statistical results (data not shown), self-reported outcomes limit the accuracy of this study. Fourth, the potential influence of medication on plasma testosterone level and on CV events cannot be excluded, although statistical adjustment for each class of drugs did not affect the results. For instance, beta-blockers have been reported to decrease plasma testosterone [27], but were taken by only nine subjects and were not related to testosterone level in our population (data not shown). Fifth, active forms of testosterone such as bioavailable and calculated free testosterone were not measured, because a direct assay of bioavailable testosterone or an assay of sex hormone binding globulin, which is necessary for free testosterone calculation, is not available in Japan. However, since previous longitudinal studies [11,12] have shown an association of total testosterone with CV mortality, the fundamental findings might not have differed if active forms of testosterone had been analyzed.

In summary, a low plasma testosterone level was associated with CV events in middle-aged Japanese men, independent of coronary risk factors and endothelial function. This study is the first to show the relationship between endogenous testosterone and CV events in Asian population, and provides evidence supporting the protective role of endogenous testosterone in the development of CV disease in men.

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References

[1] Muller M, den Tonkelaar I, Thijssen JH, Grobbee DE, van der Schouw YT. Endogenous sex hormones in men aged 40–80 years. Eur J Endocrinol 2003;149:583–9.
[2] Kaufman JM, Vermeulen A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev 2005;26:833–76.
[3] Fukai S, Akishita M, Yamada S, et al. Association of plasma sex hormone levels with functional decline in elderly men and women. Geriatr Gerontol Int 2009;9:282–9.
[4] Hak AE, Witteman JC, de Jong FH, et al. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. J Clin Endocrinol Metab 2002;87:3632–9.
[5] Simon D, Charles MA, Nahoul K, et al. Association between plasma total testosterone and cardiovascular risk factors in healthy adult men: The Telecom Study. J Clin Endocrinol Metab 1997;82:682–5.
[6] Fogari R, Preti P, Zoppi A, et al. Serum testosterone levels and arterial blood pressure in the elderly. Hypertens Res 2005;28:625–30.
[7] Stellato RK, Feldman HA, Hamdy O, Horton ES, McKinlay JB. Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts male aging study. Diabetes Care 2000;23:490–4.
[8] Akishita M, Hashimoto M, Ohike Y, et al. Low testosterone level is an independent determinant of endothelial dysfunction in men. Hypertens Res 2007;30:1029–34.
[9] van den Beld AW, Bots ML, Janssen JA, et al. Endogenous hormones and carotid atherosclerosis in elderly men. Am J Epidemiol 2003;157:25–31.
[10] Shores MM, Matsumoto AM, Sloan KL, Kivlahan DR. Low serum testosterone and mortality in male veterans. Arch Intern Med 2006;166:1660–5.

[11] Khaw KT, Dowsett M, Folkerd E, et al. Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men: European prospective investigation into cancer in Norfolk (EPIC-Norfolk) Prospective Population Study. Circulation 2007;116:2694–701.
[12] Laughlin GA, Barrett-Connor E, Bergstrom J. Low serum testosterone and mortality in older men. J Clin Endocrinol Metab 2008;93:68–75.
[13] Ogihara T, Kikuchi K, Matsuoka H, et al. The Japanese Society of Hypertension guidelines for the management of hypertension (JSH 2009). Hypertens Res 2009;32:3–107.
[14] Teramoto T, Sasaki J, Ueshima H, et al. Japan Atherosclerosis Society (JAS) Committee for epidemiology and clinical management of atherosclerosis. Diagnostic criteria for dyslipidemia. Executive summary of Japan Atherosclerosis Society (JAS) guideline for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese. J Atheroscler Thromb 2007;14:155–8.
[15] Kuzuya T, Nakagawa S, Satoh J, et al. Committee of the Japan Diabetes Society on the diagnostic criteria of diabetes mellitus. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. Diabetes Res Clin Pract 2002;55:65–85.
[16] Kobayashi K, Akishita M, Yu W, et al. Interrelationship between non-invasive measurements of atherosclerosis; flow-mediated dilation of brachial artery, carotid intima-media thickness and pulse wave velocity. Atherosclerosis 2004;173:13–8.
[17] Feldman HA, Johannes CB, McKinlay JB, Longcope C. Low dehydroepiandrosterone sulfate and heart disease in middle-aged men: cross-sectional results from the Massachusetts Male Aging Study. Ann Epidemiol 1998;8:217–28.
[18] Jeppesen LL, Jorgensen HS, Nakayama H, et al. Decreased serum testosterone in men with acute ischemic stroke. Arterioscler Thromb Vasc Biol 1996;16:749–54.
[19] Araujo AB, Kupelian V, Page ST, et al. Sex steroids and all-cause and cause-specific mortality in men. Arch Intern Med 2007;167:1252–60.
[20] Smith GD, Ben-Shlomo Y, Beswick A, et al. Cortisol, testosterone, and coronary heart disease: prospective evidence from the Caerphilly study. Circulation 2005;112:332–40.
[21] Feldman HA, Johannes CB, Araujo AB, et al. Low dehydroepiandrosterone and ischemic heart disease in middle-aged men: prospective results from the Massachusetts Male Aging Study. Am J Epidemiol 2001;153:79–89.
[22] Arnlov J, Pencina MJ, Amin S, et al. Endogenous sex hormones and cardiovascular disease incidence in men. Ann Intern Med 2006;145:176–84.
[23] Emmelot-Vonk MH, Verhaar HJ, Nakhai Pour HR, et al. Effect of testosterone supplementation on functional mobility, cognition, and other parameters in older men: a randomized controlled trial. JAMA 2008;299:39–52.
[24] Ong PJ, Patrizi G, Chong WC, et al. Testosterone enhances flow-mediated brachial artery reactivity in men with coronary artery disease. Am J Cardiol 2000;85:269–72.
[25] Tharp DL, Masseau I, Ivey J, Ganjam VK, Bowles DK. Endogenous testosterone attenuates neointima formation after moderate coronary balloon injury in male swine. Cardiovasc Res 2009;82:152–60.
[26] Jones TH, Saad F. The effects of testosterone on risk factors for, and the mediators of, the atherosclerotic process. Atherosclerosis 2009 April 24 [Epub ahead of print].
[27] Fogari R, Preti P, Derosa G, et al. Effect of antihypertensive treatment with valsartan or atenolol on sexual activity and plasma testosterone in hypertensive men. Eur J Clin Pharmacol 2002;58:177–80.

201115006B（別刷 その2）

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長寿科学総合研究事業報告書

運動器の不安定性に関与する姿勢と中枢制御機能に着目した転倒予防ガイドライン策定研究

（課題番号：Ｈ２１－長寿－一般－００５）

H21－H23 年度　総合研究報告書　別刷その２

研究代表者　鳥羽　研二

平成 24 年(2012)　3 月

〈委員会報告〉

老年病専門医の副作用経験と処方態度に関するNHKとの共同アンケート調査（高齢者薬物療法のガイドライン作成のためのワーキンググループ委員会報告）

秋下　雅弘[1)]　荒井　啓行[2)]　荒井　秀典[3)]　稲松　孝思[4)]　葛谷　雅文[5)]
鈴木　裕介[5)]　寺本　信嗣[6)]　水上　勝義[7)]　森本　茂人[8)]　鳥羽　研二[9)]

要　約　**目的**：日本老年医学会では，2005年に「高齢者に対して特に慎重な投与を要する薬物のリスト」を含む「高齢者の安全な薬物療法ガイドライン」を発表した．このような薬物有害反応（ADR）を減らす取り組みにはマスコミも関心を持ち，今般，同ガイドライン作成ワーキンググループとNHKは共同で，老年病専門医に対してADR経験と処方の実態を問うアンケート調査を行った．**方法**：2008年9月，学会ホームページに掲載された全ての老年病専門医（1,492名）の掲載住所宛にアンケートを郵送した．質問項目は，1）この1年間に経験した高齢者ADRの有無（他機関の処方含む），2）上記リスト薬からベンズアミド系抗精神病薬，ベンゾジアゼピン系睡眠薬，ジゴキシン（≧0.15 mg/日），ビタミンD（アルファカルシドール≧1.0 μg/日）および自由追加薬について，過去のADR経験頻度，3）ADR予防目的による薬剤の減量・中止の有無，4）課題と取り組みについての自由意見，とした．**結果**：回答数425件（29%）．1）1年間のADR；72%．2）過去のADR；ベンズアミド79%（稀に54%，よく25%，以下同），ベンゾジアゼピン86%（62%，24%），ジゴキシン70%（61%，9%），ビタミンD37%（33%，4%）．自由回答では，非ステロイド性消炎鎮痛薬が最も多く，降圧薬，抗血小板薬，抗不整脈薬，血糖降下薬，抗うつ薬が次いだ．3）ADR予防目的の減量・中止93%．4）自由意見；ADRに関する医師・患者の啓発活動，老年病専門医の養成，多剤処方回避の指針作りやシステムの確立を挙げる意見が多かった．**結語**：老年病専門医はADRをよく経験する一方，多くは予防的対策を講じている．今回の意見を，新しい指針作りや啓発活動に生かすべきである．

Key words：薬物有害作用，ガイドライン，Beers list，多剤併用，老年病専門医

（日老医誌　2009；46：271-274）

緒　言

若年者に比べて高齢者では薬物有害反応（adverse drug reactions，ADR）の発生が多く，かつ重症であることが知られる．最近報告された観察研究の系統的レビュー[1)]によると，ADRに関連した入院は若年成人の6.3%に対して高齢者では10.7%であった．本邦の調査でも，一般病院の入院症例では，60歳未満に比べて70歳以上では1.5倍以上のADR出現率を示し[2)]，高齢者の6～15%にADRを認めた[3)]．外来患者や介護施設でも高齢者の10%以上にADRがみられることが欧米では報告されている[4)]．

このように高齢者でADRの頻度が高い要因として様々なものが挙げられるが，特に，薬物代謝の加齢変化を背景とした“薬の効き過ぎ”と多剤併用（polypharmacy）が重要とされる[2)~4)]．薬効のエビデンスも限られた現状で，いかに安全性に配慮した高齢者の薬物療法を実施するべきなのか，高齢者医療の現場で使用できる指針が必要である．

日本老年医学会では，高齢者のADRを減らすべく，以前から学術集会の企画や本誌を通じて啓発活動を行ってきた．その一環として，老人医療委員会の下部に，Ad

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Survey on geriatricians' experiences of adverse drug reactions caused by potentially inappropriate medications: Commission report of the Japan Geriatrics Society
1）Masahiro Akishita：東京大学老年病科
2）Hiroyuki Arai：東北大学老年科
3）Hidenori Arai：京都大学老年内科
4）Takashi Inamatsu：東京都老人医療センター感染症科
5）Masafumi Kuzuya, Yusuke Suzuki：名古屋大学老年科
6）Shinji Teramoto：国立病院機構東京病院呼吸器科
7）Katsuyoshi Mizukami：筑波大学精神神経科
8）Shigeto Morimoto：金沢医科大学高齢医学，
以上，高齢者薬物療法のガイドライン作成のためのワーキンググループ
9）Kenji Toba：杏林大学高齢医学，日本老年医学会老人医療委員長

表1　高齢者への薬剤処方についてのアンケートで用いた質問用紙

1:ここ**1年以内**に、高齢者が薬剤による副作用(有害作用)を起こした症例のご経験はありますか？
（他の医療機関で処方を受けた患者を含みます）

□ ある　　□ ない

※ここからは、過去にご経験された症例全体についてお伺いします。
前項で「ない」と答えられた先生も、それ以前にご経験があればお答えください。

2: 副作用は、どのような薬剤で起きることが多いですか？該当の□にチェックを入れて下さい。
※薬剤の名称などに関しては、別紙の「高齢者に対して特に慎重な投与を要する薬物のリスト」をご参照下さい

No		頻度		
		全く無い	まれに	よくある
1	抗精神病薬(ベンズアミド系) ※添付リスト参照	□	□	□
2	睡眠薬(ベンゾジアゼピン系) ※添付リスト参照	□	□	□
3	強心配糖体 ※添付リスト参照	□	□	□
4	ビタミンD剤 ※添付リスト参照	□	□	□
5	(自由記述)	□	□	□
6	(自由記述)	□	□	□
7	(自由記述)	□	□	□

3:こうした副作用を伴う薬剤処方に関して、どのような課題や背景があると思いますか？

4:副作用への対策として、症状が出ていなくても、薬剤を予防的に減量・中止することはありますか？

□ ある　　□ ない

5:高齢者に安全な薬物療法を行うために、今後どのような取り組み・施策が有効だと考えられますか？

ご協力、本当にありがとうございました。

hoc Committee「高齢者薬物療法のガイドライン作成のためのワーキンググループ」が2003年に結成され，2005年には高齢者のADRを減らすための指針である「高齢者の安全な薬物療法ガイドライン2005」[5]を発表した．その中で，ADRの危険性が高い，あるいはより安全な代替薬が存在すると判断された45種類の薬剤（群）を「高齢者に対して特に慎重な投与を要する薬物のリスト」として選定し，学会ホームページにも発表している．このリストは，欧米で使われているBeersリスト[6)7)]の日本版であり，多剤併用の回避とADRの予防を目的として，高齢者医療の現場や介護施設で使用することができる．

こういった活動はマスコミからも注目されているが，今後さらに日本老年医学会として高齢者の薬物療法に関する提言を出し，国民に啓発していくべきだと考えられる．そのためにはメディアを活用することが重要であり，今般NHK（日本放送協会）の番組で高齢者のADRを取り上げるに際し，高齢者薬物療法のガイドライン作成のためのワーキンググループとNHKは共同で，2008年9月に老年病専門医に対してADR経験と処方の実態を問うアンケート調査を行ったので，その結果を報告する．

方　法

アンケートの送付と回収

2008年9月初めの時点で，日本老年医学会のホームページに掲載されていた老年病専門医1,492名全員に対し，掲載住所宛にNHKの封筒を用いてアンケートを郵送した．同封した依頼文は「高齢者への薬剤処方」に関するアンケートと題し，簡単な背景と調査の目的，老年病専門医に対するNHKと日本老年医学会との共同調査である旨を記し，FAXによる無記名の回答を求めた．FAXの回収と集計はNHKで行い，2008年9月末で締め切った．

質問項目

質問用紙を表1に示すが，1）過去1年間に経験した高齢者のADR（他機関の処方含む）の有無，2)「高齢者に対して特に慎重な投与を要する薬物のリスト」を添付し，そのうちベンズアミド系抗精神病薬（スルピリド，スルトプリド），ベンゾジアゼピン系睡眠薬（フルラゼパム，ハロキサゾラム，クアゼパム，トリアゾラム），ジゴキシン（≧0.15 mg/日），ビタミンD（アルファカルシドール≧1.0 μg/日）および自由追加薬について，過去のADR経験頻度，3）ADR予防目的による薬剤の減量・中止の有無，4）課題と取り組みについての自由意見を尋ねた．2）の4系統薬は，リストの中でも高齢者に対する処方が比較的多いと考えて選定した．

結　果

回答は425件あり，回答率28.5%であった（宛先住所不明のため返送された30件を除くと，回答率は29.1%）．

集計結果を表2に示す．過去1年以内のADR経験については，この質問に無回答の者(n=7)を入れても70%の専門医が経験ありと答えた．次に，時期を問わず尋ねた結果，ベンズアミド系抗精神病薬とベンゾジアゼピン系睡眠薬によるADRは，約1/4の専門医がよくあると回答した．これら2系統薬およびジゴキシンについては，7割～8割の回答者がADRを経験し，ビタミンDによるADRも4割近くが経験していた．また，過去1年のADR経験の有無および4系統薬によるADRの経験頻度には，いずれの間にもχ^2検定で有意な関連がみられ（データ示さず），特定の薬剤に限らず全般的にADR経験の多い老年病専門医と少ない専門医がいると考えられた．自由回答では，非ステロイド性消炎鎮痛薬の記入が最も多く，何らかの薬剤を記入した回答者240名のうち25%がよくある（よく＋稀に39%）と答えた．次いで，

表2　老年病専門医による高齢者の薬物有害反応（ADR）経験とADR予防目的の減薬態度（n = 425）

1. この1年間にADR経験あり（n = 418）	71.5%		
2. 過去のADR経験（リストの4系統薬）			
	よくある	稀にある	よく＋稀にある
1）ベンズアミド系抗精神病薬（n = 381） （スルピリド，スルトプリド）	93名 (24.4%)	207名 (54.3%)	300名 (78.7%)
2）ベンゾジアゼピン系睡眠薬（n = 386） （フルラゼパム，ハロキサゾラム，クアゼパム，トリアゾラム）	93名 (24.1%)	241名 (62.4%)	334名 (86.5%)
3）ジゴキシン≧0.15 mg/日（n = 382）	33名 (8.6%)	234名 (61.3%)	267名 (69.9%)
4）ビタミンD（n = 373） （アルファカルシドール≧1.0 μg/日）	14名 (3.7%)	125名 (33.5%)	139名 (37.3%)
3. 過去のADR経験（自由追加薬；n = 240）			
	よくある	稀にある	よく＋稀にある
1）非ステロイド性消炎鎮痛薬	60件	34件	94件
2）降圧薬	19件	27件	46件
3）抗血小板薬	17件	21件	38件
4）経口血糖降下薬	19件	15件	34件
5）抗不整脈薬	13件	17件	30件
6）抗うつ薬	15件	10件	25件
7）抗パーキンソン病薬	9件	12件	21件
8）ワルファリン	6件	7件	13件
4. ADR予防目的に薬剤を減量・中止することあり（n = 417）	93.0%		

括弧内nは各質問に対する回答数を表す．
ADR経験の自由追加薬は，系統別に10件以上の記入があったものを記す．

降圧薬（よく＋稀に19%，以下同），抗血小板薬（16%），経口血糖降下薬（14%），抗不整脈薬（13%），抗うつ薬（10%）の順であった．

処方態度についての質問では，9割以上がADRを予防する目的で薬剤を減量・中止することがあると答えた（表2）．

課題と取り組みに関する自由記述は，1）高齢者の薬物代謝およびADRのリスクに対する医師・患者の理解不足とそれに対する啓発活動の必要性，2）高齢者薬物療法の原則を理解し，総合的に診て処方薬の調整も上手くできるという点で期待される老年病専門医の養成，3）服薬過誤（飲み忘れ，重複服用）や併科受診による薬剤情報の欠如が問題で，それに対処できる服薬管理システムや薬局との連携体制の構築，4）多剤処方の根底には，患者の話をよく聞くより薬を処方する方が手っ取り早いという診療報酬制度の問題があり，それを回避するガイドラインや医療体制の確立が必要である，といった意見に集約された．

考　察

今回のアンケートでは，老年病専門医の約3割から回答が得られ，その約7割は1年間に薬物有害作用を経験する一方で，9割以上が予防的減薬を行っていると答えた．

「高齢者に対して特に慎重な投与を要する薬物のリスト」を選定する際に，2003年にワーキンググループから日本老年医学会代議員六百数十名と全国老人保健施設協会会員施設二千数百名に対して行った郵送アンケート（今回と同様にFAX返信）では，それぞれ34%と20%の回答率であった．今回のアンケートはNHKから送付する形を取ったにも関わらず29%と高い回答率が得られ，高齢者薬物療法に対する老年病専門医の関心の高さを表していると考える．

ADRについての質問は，多くの専門医が経験したと答えたが，その数字の評価は慎重に行うべきである．まず，ADRの経験が多く，ADRへの意識の高い専門医ほど回答を寄せた結果，過大評価されている可能性が否定できない．次に，重症度や因果関係などADRの判断に個人差があるし，ADRの経験も記録ではなく記憶に基づいた点も精度には問題がある．特に個々の薬剤については，頻度分類に定義も付けずに（全く無い・まれに・よくある）から選んでもらったが，後2者のどちらを選択するかは個人の印象に依存するところが大きい．また，ADRは当然処方頻度とも関連するので，自由追加薬で非ステロイド性消炎鎮痛薬や降圧薬といった高齢者でよく処方される薬剤を挙げる回答が多いことに繋がったの

かもしれない．

このように，今回のアンケート調査は，正確なADRの発生状況や個々の薬剤使用によるADR発生率を把握するものではない．高齢者医療のエキスパートである老年病専門医がADRをどのように考えているかを集計することが目的であり，頻度分類も主観的であるが故に問題意識を反映した評価になったとも言える．そのように考えると，7～8割の回答者がADRを経験しているベンズアミド系抗精神病薬，ベンゾジアゼピン系睡眠薬，ジゴキシンが「高齢者に対して特に慎重な投与を要する薬物のリスト」に入っていることは妥当であろう．今回，リスト薬からビタミンD（アルファカルシドール≧1.0 μg/日）についても尋ねた．この薬剤は欧米のリスト[6)~8)]には含まれていないが，骨粗鬆症に対して漫然と高用量が，しかもカルシウム製剤とよく併用され，結果的に高カルシウム血症を来す高齢者をしばしば経験するためリストに加えられた．ビタミンDによるADRの経験は37％という今回の調査結果からも，リストに入れておくことは妥当と思われる．ADRの経験が多いとして自由回答に10件以上記入された薬剤は，ワルファリンを除いて，同系統薬のいずれかが「高齢者に対して特に慎重な投与を要する薬物のリスト」に含まれている．リストに含まれていない薬剤で多く回答が寄せられたものについては，リストの改訂に際して検討が必要である．

老年病専門医の処方態度として，ADRの予防目的に薬剤を減量・中止することがある者の割合が93％にも上った結果は評価に値する．日本老年医学会による教育活動の成果であり，老年病専門医の先進性の表れかもしれない．改めてADRに関する啓発と今回のアンケート結果のような情報の提示が必要と思われる．

何割の症例に減薬介入が行われているかのデータは未だない．大学病院老年科5施設の入院症例で行ったADR調査[3)]のデータを解析し直すと，入退院時の服薬数記録がある1,002例のうち20％で処方薬剤数が減少していた．追加薬も含まれるので実際の減薬率はもっと高いはずであるが，いずれにしても減薬理由は不明である．老人保健施設入所者の調査[9)]では，服薬のある581例のうち230例（40％）で入所後に何らかの薬剤削減が行われていた．特に1997年版Beersリスト[6)]の該当薬が61件から41件へ33％も減少していたことは，意図的な選択でないにしても，特筆するべきであろう．今後は，ADRの予防目的に行われる減薬の実態調査（症例単位）と減薬介入の効果を検証する大規模試験，できれば無作為比較試験が実施されるべきである．

最後に，自由記述で様々な意見をいただいた．日常診療で感じている諸問題や提言は納得できるものばかりで，多数意見は結果に述べたように要約できる．また，患者の“薬物依存”と“薬物恐怖”，有効性に偏った製薬会社の宣伝，高齢者の個人差を無視した臓器別診療ガイドラインなどがADRの増加や不適切な服薬に結びついていると指摘する意見も相当数あった．今後，これらの経験や意見を元に，調査研究を積み重ねて，新しい指針作りや啓発活動を行っていくことが重要と考えられた．

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文　献

1) Kongkaew C, Noyce PR, Ashcroft DM: Hospital admissions associated with adverse drug reactions: a systematic review of prospective observational studies. Ann Pharmacother 2008; 42: 1017–1025.
2) 鳥羽研二，秋下雅弘，水野有三，江頭正人，金　承範，阿古潤哉ほか：日老医誌　1999; 36: 181–185.
3) 秋下雅弘，寺本信嗣，荒井秀典，荒井啓行，水上勝義，森本茂人ほか：高齢者薬物療法の問題点：大学病院老年科における薬物有害作用の実態調査．日老医誌　2004; 41: 306–306.
4) Rothschild JM, Bates DW, Leape LL: Preventable medical injuries in older patients. Arch Intern Med 2000; 160: 2717–2728.
5) 高齢者の安全な薬物療法ガイドライン2005.（日本老年医学会編），メジカルビュー社，東京，2005.
6) Beers MH: Explicit criteria for determining potentially inappropriate medication use by the elderly. An update. Arch Intern Med 1997; 157: 1531–1536.
7) Fick DM, Cooper JW, Wade WE, Waller JL, Maclean JR, Beers MH: Updating the Beers criteria for potentially inappropriate medication use in older adults: results of a US consensus panel of experts. Arch Intern Med 2003; 163: 2716–2724.
8) McLeod PJ, Huang AR, Tamblyn RM, Gayton DC: Defining inappropriate practices in prescribing for elderly people: a national consensus panel. CMAJ 1997; 156: 385–391.
9) Mita Y, Akishita M, Tanaka K, Yamada S, Nakai R, Tanaka E, et al.: Improvement of inappropriate prescribing and adverse drug withdrawal events after admission to long-term care facilities. Geriatr Gerontol Int 2004; 4: 146–150.

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PRE-CLINICAL RESEARCH

Sirolimus and Everolimus Induce Endothelial Cellular Senescence Via Sirtuin 1 Down-Regulation

Therapeutic Implication of Cilostazol After Drug-Eluting Stent Implantation

Hidetaka Ota, MD, PHD, Masato Eto, MD, PHD, Junya Ako, MD, PHD, Sumito Ogawa, MD, PHD, Katsuya Iijima, MD, PHD, Masahiro Akishita, MD, PHD, Yasuyoshi Ouchi, MD, PHD

Tokyo, Japan

Objectives The aim of this study was to compare the effects of paclitaxel, sirolimus, and everolimus on the senescent phenotype in human endothelial cells, and to further investigate possible involvement of mammalian sirtuin 1 (Sirt1) down-regulation as a mechanism.

Background Endothelial cell senescence may play a role in impaired re-endothelialization after drug-eluting stent (DES) implantation. Recently, the down-regulation of Sirt1 has been shown to mediate oxidative stress-induced endothelial senescence.

Methods Senescent human umbilical vein endothelial cells (HUVEC) were judged by senescence-associated β-galactosidase assay (SA-βgal), morphological appearance, and plasminogen activator inhibitor (PAI)-1.

Results Treatment with paclitaxel, sirolimus, and everolimus significantly caused a senescent phenotype and PAI-1 up-regulation, associated with a decrease in endothelial nitric oxide synthase (eNOS) and Sirt1 expression. Overexpression of Sirt1 or Sirt1 activation reversed the sirolimus- or everolimus-induced senescent phenotype. Interestingly, paclitaxel-induced senescence was not suppressed by Sirt1 overexpression, suggesting the existence of a different mechanism. Cilostazol markedly inhibited the sirolimus- or everolimus-induced senescent phenotype (sirolimus or everolimus [2.5 nmol/l]; 49.2% or 53.0% SA-βgal positive vs. only 13.6% or 14.6% with cilostazol [100 μmol/l]) and PAI-1 up-regulation, but had no influence on the effects of paclitaxel. Finally, aspirin significantly blunted sirolimus- or everolimus-induced senescence, but neither ticlopidine nor clopidogrel had any effects.

Conclusions Sirolimus and everolimus induce endothelial senescence involving down-regulation of Sirt1. In contrast, the development of endothelial senescence by paclitaxel involves a Sirt1-independent pathway. Because sirolimus and everolimus are involved in Sirt1 modulation, cilostazol rescues HUVEC from sirolimus- or everolimus-induced senescence. These results may have therapeutic implications in the clinical sequelae after DES implantation. (J Am Coll Cardiol 2009;53:2298–305) © 2009 by the American College of Cardiology Foundation

Because of the marked reduction in restenosis rate, drug-eluting stent (DES) implantation has been the mainstay of percutaneous coronary interventions. Recent real-world experiences, however, have raised concern over a possible increase in the frequency of late stent thrombosis (ST), occurring more than 1 year after stent implantation (1). Pathological studies have shown that a lack or delay of endothelial healing plays an important role in the pathogenesis of ST (2).

From the Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan. Supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Culture, and Sports of Japan (20249041, 18590801, 18890056).

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Endothelial senescence has been proposed to be involved in endothelial dysfunction, atherogenesis, and thrombosis (3). Drugs used for DES, including paclitaxel and limus family members (e.g., sirolimus, everolimus), inhibit the growth of endothelial cells, possibly leading to endothelial senescence and delayed re-endothelialization. However, the effect of such drugs on endothelial cell senescence has not been well documented.

Mammalian sirtuin 1 (Sirt1), the closest homolog of silent information regulator 2, regulates the cell cycle, senescence, apoptosis, and metabolism by interacting with a number of molecules, including p53, promyelocytic leukemia protein, and peroxisome proliferators-activated receptor γ (4–6). Sirt1 has been recognized as a key regulator of vascular endothelial homeostasis controlling

angiogenesis, endothelial senescence, and dysfunction (7–9). Recently, we reported that an antiplatelet drug, cilostazol, inhibited oxidative stress-induced endothelial senescence through up-regulation of Sirt1 (10).

The aims of this study were to compare the effects of paclitaxel, sirolimus, and everolimus on the senescent phenotype in human endothelial cells and to further investigate the possible involvement of Sirt1 down-regulation as a mechanism. Potential protective effects of cilostazol and other widely used antiplatelet drugs (aspirin, ticlopidine, and clopidogrel) were also investigated.

Methods

Materials. Sirolimus and resveratrol were purchased from Wako Chemical Industries, Ltd. (Tokyo, Japan). Aspirin, ticlopidine, clopidogrel, paclitaxel, and everolimus were from Sigma (St. Louis, Missouri). 8-hydroxydeoxyguanosine (8-OHdG, AB5830) was from Chemicon (Temecula, California). Cilostazol was provided by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan).

Cell culture. Human umbilical vein endothelial cells (HUVEC) were purchased from Cambrex (Walkersville, Maryland), and maintained in endothelial growth medium (EGM-2) (EGM-2 single-quots, Cambrex). Population doubling levels were calculated as described previously (11), and all experiments were performed at population doubling level of 8 to 9.

Transform from ticlopidine and clopidogrel into liver metabolites. Human liver microsomes were purchased from BD Gentest (Woburn, Massachusetts). As described by Savi et al. (12), liver microsomes were adjusted to 0.75 mg protein/ml in 100 mmol/l potassium phosphate buffer, pH 7.4, containing 100 mmol/l potassium fluoride and 10 mmol/l glutathione. Ticlopidine and clopidogrel were added at a final concentration of 1 mmol/l, and the reaction was initiated with 1 mmol/l β-nicotinamide adenine dinucleotide phosphate. Incubation was carried out at 37°C under continuous stirring (100 rpm) and protection from light using a reciprocal incubator. After 60 min, the incubation medium was cooled to 4°C and centrifuged at 10,000 *g* for 10 min, and supernatants were collected.

Senescence-associated β-galactosidase (SA-βgal) assay. The HUVEC were grown in 100-mm collagen-coated dishes to 80% confluence. They were pre-treated with vehicle (0.05% dimethylsulfoxide), cilostazol (10, 30, 100

Abbreviations and Acronyms

DES = drug-eluting stent(s)
EGM = endothelial growth medium
eNOS = endothelial nitric oxide synthase
HUVEC = human umbilical vein endothelial cells
PAI = plasminogen activator inhibitor
SA-βgal = senescence-associated β-galactosidase assay
SES = sirolimus-eluting stent(s)
Sirt1 = silent mating type information regulation 2 homolog 1
ST = stent thrombosis

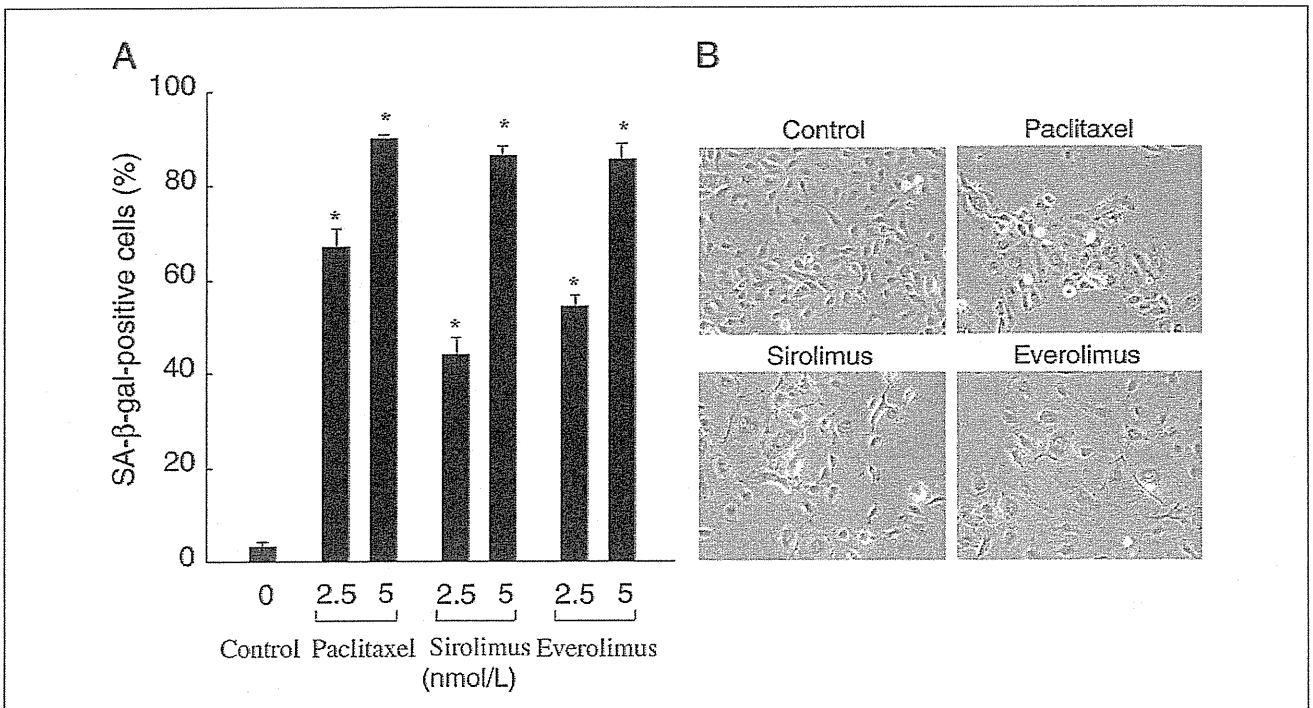

Figure 1 Paclitaxel, Sirolimus, and Everolimus Induced a Premature Senescent Phenotype

Paclitaxel, sirolimus, and everolimus (2.5, 5 nmol/l) induced a premature senescent phenotype in HUVEC as judged by SA-βgal **(A)** (unpaired *t* test, *p < 0.05 vs. control, n = 3) and morphological changes **(B)**. HUVEC = human umbilical vein endothelial cells; SA-βgal = senescence-associated β-galactosidase assay.

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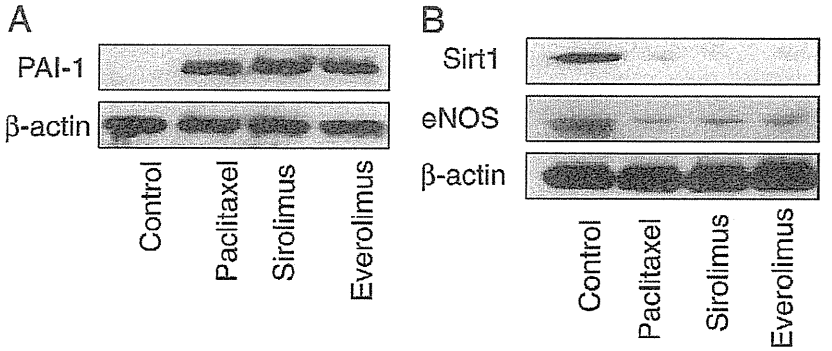

Figure 2 Expression of PAI-1, eNOS, and Sirt1 After Treatment With Paclitaxel, Sirolimus, or Everolimus

Expression of PAI-1 **(A)**, eNOS, and Sirt1 **(B)** at 10 days after treatment with paclitaxel, sirolimus, or everolimus (2.5 nmol/l) were analyzed by Western blotting. Whole-cell lysates (20 μg) were prepared from treated HUVEC. Similar results were observed in 3 independent experiments. eNOS = endothelial nitric oxide synthase; PAI-1 = plasminogen activator inhibitor-1; Sirt1 = silent mating type information regulation 2 homolog 1.

μmol/l), aspirin (10, 30, 100 μmol/l), ticlopidine (10, 30, 100 μmol/l), clopidogrel (10, 30, 100 μmol/l), or resveratrol (10, 30, 100 μmol/l) diluted in EGM-2 medium for 24 h. Nitric oxide production was induced with calcium A 23187 ionophore (Sigma, 1 μmol/l) followed under incubation with aspirin, cilostazol, ticlopidine, or clopidogrel for 20 min (n = 3 samples in each experiment). The HUVEC were washed 3 times with EGM-2 and then treated for 24 h

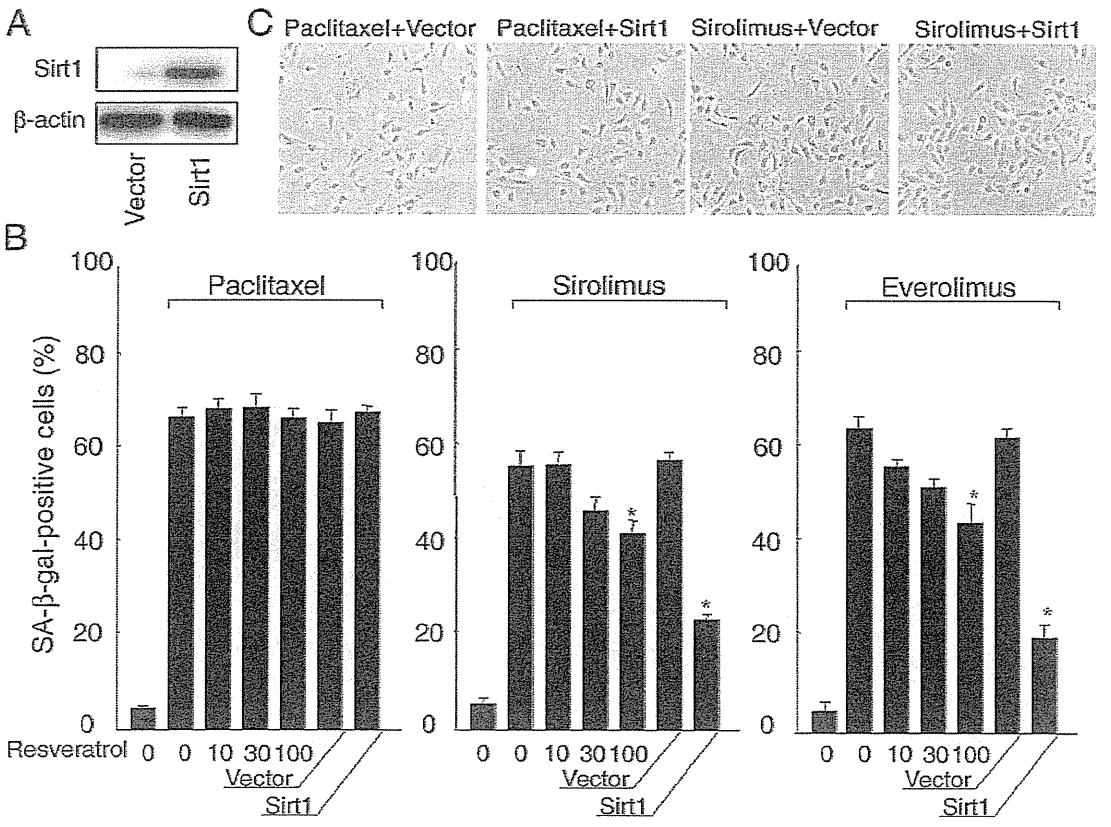

Figure 3 Overexpression of Sirt1 Inhibited Sirolimus- and Everolimus-Induced Senescent Phenotype

Overexpression of Sirt1 **(A)** or treatment with resveratrol (10, 30, 100 μmol/l) inhibited sirolimus- and everolimus-induced senescent phenotype as judged by SA-βgal **(B)** (unpaired *t* test, *p < 0.05 vs. control, n = 3) and morphological changes **(C)**. Abbreviations as in Figures 1 and 2.

with paclitaxel, sirolimus, or everolimus (5 nmol/l) diluted in EGM-2. After the treatment, HUVEC were trypsinized, reseeded at a density of 1×10^5 in 60-mm dishes, and cultured in EGM-2 containing these compounds (cilostazol, aspirin, ticlopidine, or clopidogrel) for 10 days. At 10 days after treatment with paclitaxel, sirolimus, or everolimus, HUVEC were fixed and the proportion of SA-βgal positive cells was determined as described by Dimri et al. (13).

Sirt1 overexpression. The Sirt1 was overexpressed by transfection with pIRES-Sirt1 (10 μg), which was provided by Dr. R. A. Weinberg (4,14), using jetPEI-HUVEC (Polyplustransfection, Illkirch, France) according to the manufacturer's instructions. At 3 days after transfection, HUVEC were treated with sirolimus, everolimus, or paclitaxel (2.5 nmol/l) for 24 h, washed 3 times with medium, and cultured for up to 10 days.

Immunoblotting. Cells were lysed on ice for 1 h in buffer (50 mmol/l Tris-HCl, pH 7.6, 150 mmol/l NaCl, 1% NP-40, 0.1% sodium dodecyl sulfate [SDS], 1 mmol/l dithiothreitol, 1 mmol/l sodium vanadate, 1 mmol/l phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 mmol/l sodium fluoride). Equal amounts of protein were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. After blocking, the filters were incubated with the following antibodies; anti-endothelial nitric oxide synthase (eNOS) (BD Transduction Laboratories, Franklin Lakes, New Jersey), anti-Sirt1 (Santa Cruz Biotechnology, Inc., Santa Cruz, California), anti-plasminogen activator inhibitor-1 (PAI-1) (Molecular Innovations, Inc.) and anti–β-actin (Sigma). After washing and incubation with horseradish peroxidase-conjugated antirabbit or antimouse immunoglobulin G (Amersham, Piscataway, New Jersey) for 1 h, the antigen–antibody complexes were visualized using an enhanced chemiluminescence system (Amersham).

Data analysis. Values are shown as mean ± SEM in the text and figures. Unpaired Student *t* test was used for statistical analysis. Probability values <0.05 were considered significant.

Results

Paclitaxel, sirolimus, and everolimus induce premature senescence in human endothelial cells. To investigate the effect of paclitaxel, sirolimus, and everolimus on the endothelial senescent phenotype, we treated HUVEC with each drug for 24 h. Treatment with paclitaxel, sirolimus, and everolimus significantly induced a senescent phenotype as judged by SA-βgal and an enlarged, flattened cell morphological appearance at 10 days (Figs. 1A and 1B). Of note, paclitaxel had a tendency to arrest senescent growth, associated with round colony formation, because it may have the ability to stabilize microtubules and inhibit cell division.

Effects of paclitaxel, sirolimus, and everolimus on PAI-1, eNOS, and Sirt1 expression in human endothelial cells. Plasminogen activator inhibitor-1 is a potent inhibitor of fibrinolysis and a mediator of thrombosis, and its expression can also be used as a marker of endothelial senescence (15). In parallel with SA-βgal, expression of PAI-1 was increased by treatment with paclitaxel, sirolimus, and everolimus (Fig. 2A). Both eNOS and Sirt1 play a pivotal role in endothelial function and senescence (8–16); therefore, we examined

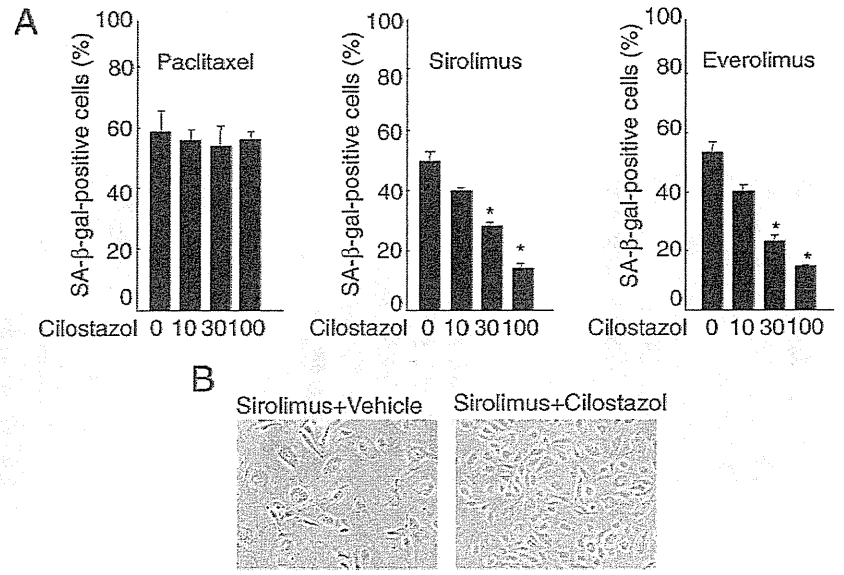

Figure 4 Effect of Cilostazol on Paclitaxel-, Sirolimus-, or Everolimus-Induced Senescent Phenotype

Effect of cilostazol (10, 30, 100 μmol/l) on paclitaxel-, sirolimus-, or everolimus (2.5 nmol/l)-induced senescent phenotype as judged by SA-βgal **(A)** (unpaired *t* test, *p < 0.05 vs. control, n = 3) and morphological changes (sirolimus) **(B)**. Abbreviations as in Figure 1.

their expression. As shown in Figure 2B, paclitaxel, sirolimus, and everolimus decreased the expression of eNOS and Sirt1.

Overexpression of Sirt1 and resveratrol inhibited sirolimus- and everolimus-induced senescent phenotype. To clarify the molecular mechanisms of regulation of endothelial senescence induced by paclitaxel, sirolimus, and everolimus, we induced Sirt1 gene overexpression and performed treatment with resveratrol, a Sirt1 chemical activator. We confirmed overexpression of Sirt1 at the protein level (Fig. 3A). As shown in Figures 3B and 3C, overexpression of Sirt1 and resveratrol decreased SA-βgal–positive cells and specific morphological senescent changes induced by sirolimus and everolimus. Unexpectedly, paclitaxel-treated cells could not be restored from a senescent phenotype. These results indicate that Sirt1 is a key molecule in the modulation of sirolimus- and everolimus- but not paclitaxel-induced senescence of HUVEC.

Cilostazol inhibits sirolimus- or everolimus-induced premature senescence. We examined the effect of cilostazol on a senescent phenotype in human endothelial cells. We pre-treated HUVEC with cilostazol for 24 h before the addition of paclitaxel, sirolimus, or everolimus. Treatment with cilostazol inhibited the senescent phenotype induced by sirolimus or everolimus at 10 days (Fig. 4A). Under treatment with sirolimus or everolimus (2.5 nmol/l), 49.2% or 53.0% of cells were SA-βgal positive, versus only 13.6% or 14.6% of cilostazol (100 μmol/l)-treated cells under the same conditions. Treatment with cilostazol decreased the specific senescent morphological changes and expression of PAI-1 induced by sirolimus and everolimus as well (Figs. 4B and 5A). Human umbilical vein endothelial cells treated with paclitaxel did not show a reduction of the senescent phenotype by treatment with cilostazol (Figs. 4A and 5A). At the same time, we checked the effect of other antiplatelet drugs (aspirin, ticlopidine, and clopidogrel) on the senescent phenotype (Figs. 5A and 6). Treatment with aspirin decreased SA-βgal–positive cells and expression of PAI-1, but treatment with ticlopidine or clopidogrel did not have any effect on them. To evaluate potential clinical interactions of ticlopidine and clopidogrel on endothelial senescence, we next used these compounds after incubation with human hepatic microsomes. Treatment with metabolites of ticlopidine and clopidogrel did not decrease the paclitaxel-, sirolimus-, or everolimus-induced senescent phenotype (paclitaxel, sirolimus, everolimus [2.5 nmol/l]; 61.4%, 55.7%, or 53.5% SA-βgal positive vs. 64.9%/62.2%, 55.9%/54.6%, or 55.8%/54.7% with metabolites of ticlopidine/clopidogrel [100 μmol/l]).

Treatment with cilostazol reversed eNOS and Sirt1 expression. To explore the mechanism by which cilostazol prevented premature endothelial senescence induced by sirolimus and everolimus, we examined whether they could promote eNOS and the longevity gene, Sirt1. Cilostazol and aspirin

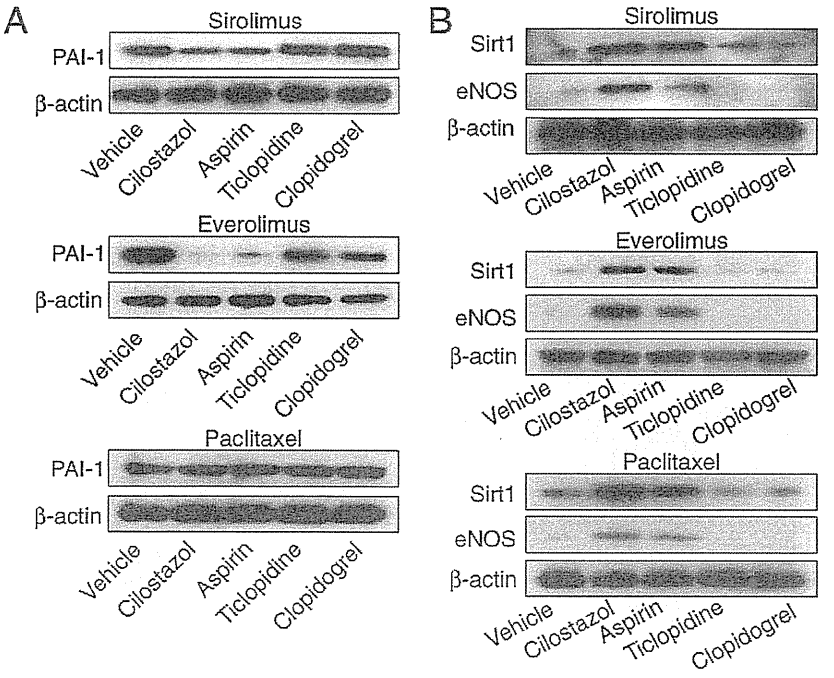

Figure 5 Expression of PAI-1, eNOS, and Sirt1 When HUVEC Were Treated With Cilostazol, Aspirin, Ticlopidine, or Clopidogrel

Expression of PAI-1 (**A**), eNOS, and Sirt1 protein (**B**) at 10 days when HUVEC were treated with cilostazol, aspirin, ticlopidine, or clopidogrel after addition of sirolimus, everolimus, or paclitaxel (2.5 nmol/l). Whole-cell lysates (20 μg) were prepared from treated HUVEC. Similar results were observed in 3 independent experiments. Abbreviations as in Figures 1 and 2.

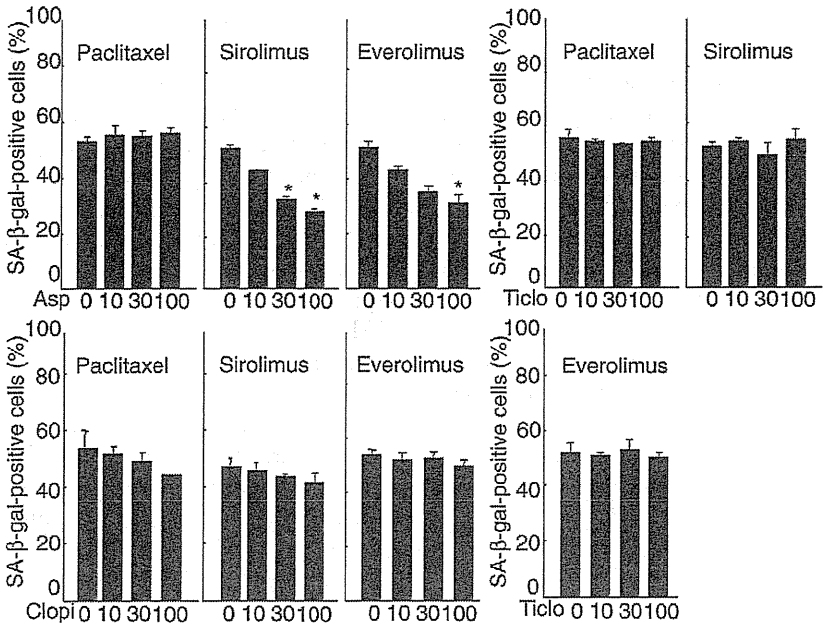

Figure 6 Effect of Aspirin, Ticlopidine, and Clopidogrel on Paclitaxel-, Sirolimus-, or Everolimus-Induced Senescent Phenotype

Effect of aspirin (asp), ticlopidine (ticlo), and clopidogrel (clopi) (10, 30, 100 μmol/l) on paclitaxel-, sirolimus-, or everolimus (2.5 nmol/l)-induced senescent phenotype as judged by SA-βgal (unpaired *t* test, *p < 0.05 vs. control, n = 3). Abbreviations as in Figure 1.

significantly increased eNOS and Sirt1 protein at 10 days after treatment with sirolimus or everolimus (Fig. 5B). Although cilostazol and aspirin did not inhibit a paclitaxel-induced senescent phenotype, it reversed eNOS and Sirt1 expression (Fig. 5B). Moreover, treatment with cilostazol or aspirin decreased 8-OHdG, a marker of oxidative stress (Fig. 7).

Discussion

In the present study, we found that paclitaxel, sirolimus, and everolimus significantly induced endothelial senescence, with decreased eNOS and Sirt1 expression. Endothelial cell senescence induced by sirolimus or everolimus was partly blunted by Sirt1 overexpression or Sirt1 activation by resveratrol, whereas senescence induced by paclitaxel occurred through a Sirt1-independent pathway.

Drug-eluting stents significantly reduce the rate of restenosis, but several pre-clinical and clinical safety concerns (17,18) related to its use have been expressed. One of the most important issues raised is ST, which is partly caused by delayed re-endothelialization. However, the pathogenesis of re-endothelialization or ST has not yet been fully explored (19), and evaluation of the degree

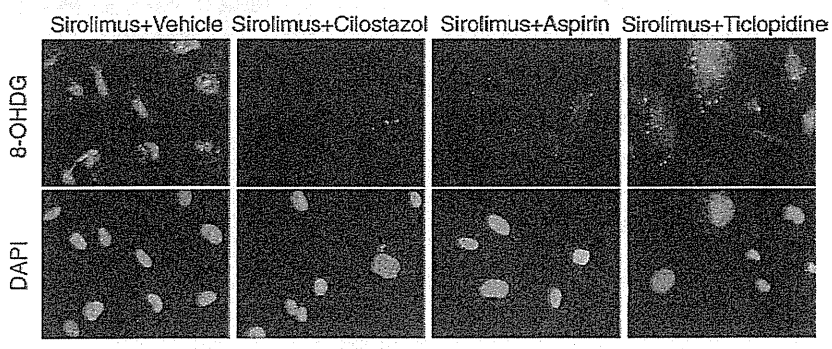

Figure 7 Antioxidative Effect of Cilostazol, Aspirin, Ticlopidine, and Clopidogrel on Sirolimus-Induced Senescent Phenotype

Effect of cilostazol, aspirin, ticlopidine, and clopidogrel (10, 30, 100 μmol/l) on sirolimus (2.5 nmol/l)-induced senescent phenotype as judged by immunofluorescent cell staining for 8-hydroxydeoxyguanosine (8-OHDG) (green) and diamidino-2-phenylindole dihydrochloride (DAPI) (nuclear staining, blue).

of endothelial senescence induced by drugs used for DES is of great significance.

First, we showed that paclitaxel, sirolimus, and everolimus significantly induced endothelial senescence, in association with reduced eNOS and increased PAI-1 expression, which are implicated in endothelial dysfunction and thrombogenesis. Similar to our results, recent studies showed that sirolimus increased tissue factor expression (1,20), and that both sirolimus and paclitaxel selectively increased PAI-1 expression (21). Secondly, we showed that overexpression of Sirt1 reversed the sirolimus- or everolimus-induced senescent phenotype. Interestingly, paclitaxel-induced senescence was not suppressed by Sirt1 overexpression. These results suggest that paclitaxel, sirolimus, and everolimus induce endothelial senescence through 2 distinct pathways, and sirolimus- and everolimus-induced senescence is mainly regulated by Sirt1. In addition to these findings, treatment with cilostazol reversed the senescent phenotype induced by sirolimus or everolimus, but not paclitaxel. As previously reported, cilostazol activates protein kinase A and PI3K/Akt signaling and increases eNOS expression (22). Recently, we reported that cilostazol inhibited oxidative stress–induced premature senescence via up-regulation of Sirt1 in human endothelial cells (10). These results imply that nitric oxide production by cilostazol increases Sirt1 expression, and increased Sirt1 expression inhibits endothelial senescence. Therefore, the eNOS–Sirt1 axis may play an important role in the modulation of sirolimus- or everolimus-induced premature senescence. Although cilostazol reversed eNOS and Sirt1 expression in HUVEC treated with paclitaxel, cilostazol did not rescue HUVEC from a senescent phenotype. Paclitaxel inhibits cell proliferation by promoting polymerization of tubulin dimers and subsequently stabilizing microtubules into an assembled state, which blocks the cell cycle from G2 to M phase. In contrast, sirolimus and everolimus bind to FKBP12 and subsequently bind to the mammalian target of rapamycin. This complex then increases cellular cyclin-dependent kinase inhibitor p27 and decreases the phosphorylation of retinoblastoma protein, which blocks the cell cycle from G1 to S phase (23). As previously reported (24), microtubules are not substrates of Sirt1. Because paclitaxel strongly stabilizes microtubules and inhibits cell division, we speculate that increased Sirt1 expression was insufficient for reversal of the paclitaxel-induced senescent phenotype, or there might be other regulators.

Resveratrol exerted a significant effect at a concentration of 100 μmol/l (Fig. 3), which seems a relatively high concentration to achieve in vivo. However, considering that novel small molecule activators of Sirt1, even 1,000-fold more potent than resveratrol, are being identified (Sirtris Pharmaceuticals Inc., Boston, Massachusetts) (25), we suggest that using these chemical agents to activate Sirt1 might be of potential therapeutic value for protection against endothelial senescence.

In the current study, the impact of cilostazol in reducing post-operative sequelae seemed more marked in sirolimus-eluting stents (SES) than in paclitaxel-eluting stents. Moreover, SES plus triple treatment reduced restenosis more than did SES plus standard dual treatment (26). Cilostazol not only is an antiplatelet drug, but also has pleiotropic effects, including a vasodilating action, endothelial cell protection, and improvement of lipid metabolism (27–29). However, the impact of cilostazol on endothelial senescence after DES implantation has not been reported. Based on this study, we emphasize that cilostazol might have favorable effects against endothelial senescence when administered with sirolimus or everolimus, through up-regulation of Sirt1. To assess the implications of our findings, further studies are needed to examine the degree of senescence as well as the expression pattern of Sirt1 in the arterial wall after deployment of DES in vivo.

Conclusions

Our study shows that paclitaxel, sirolimus, and everolimus induce endothelial senescence through 2 distinct pathways. The development of endothelial senescence induced by sirolimus and everolimus is Sirt1-dependent, whereas paclitaxel acts through a Sirt1-independent pathway. Because sirolimus and everolimus (limus family) were involved in Sirt1 modulation, cilostazol reversed sirolimus- or everolimus-induced senescence. Our results may have an interesting clinical implication that triple antiplatelet therapy has more beneficial effects on endothelial senescence than has standard dual therapy of SES and everolimus-eluting stents.

Acknowledgments

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Reprint requests and correspondence: Dr. Yasuyoshi Ouchi, Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: youchi-tky@umin.ac.jp.

REFERENCES

1. Lüscher TF, Steffel J, Eberli FR, et al. Drug-eluting stent and coronary thrombosis: biological mechanisms and clinical implications. Circulation 2007;115:1051–8.
2. Joner M, Finn AV, Farb A, et al. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. J Am Coll Cardiol 2006;48:193–202.
3. Minamino T, Miyauci H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. Circulation 2002;105:1541–4.
4. Vaziri H, Dessain SK, Ng Eaton E, et al. hSIR2 (SIRT1) functions as an NAD-dependent p53 deacetylase. Cell 2001;107:149–59.

5. Langley E, Pearson M, Faretta M, et al. Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. EMBO J 2002;21:2383–96.
6. Picard F, Kurtev M, Chung N, et al. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature 2004;429: 771–6.
7. Potente M, Ghaeni L, Baldessari D, et al. SIRT1 controls endothelial angiogenic functions during vascular growth. Genes Dev 2007;21: 2644–58.
8. Ota H, Akishita M, Eto M, Iijima K, Kaneki M, Ouchi Y. Sirt1 modulates premature senescence-like phenotype in human endothelial cells. J Mol Cell Cardiol 2007;43:571–9.
9. Potente M, Dimmeler S. Emerging roles of SIRT1 in vascular endothelial homeostasis. Cell Cycle 2008;7:2117–22.
10. Ota H, Eto M, Kano MR, et al. Cilostazol inhibits oxidative stress-induced premature senescence via up-regulation of Sirt1 in human endothelial cells. Arterioscler Thromb Vasc Biol 2008;28: 1634–9.
11. Maciag T, Hoover GA, Stemerman MB, Weinstein R. Serial propagation of human endothelial cells in vitro. J Cell Biol 1981;91:420–6.
12. Savi P, Pereillo JM, Uzabiaga MF, et al. Identification and biological activity of the active metabolite of clopidogrel. Thromb Haemost 2000;84:891–6.
13. Dimri GP, Lee X, Basile G, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci U S A 1995;92:9363–7.
14. Matsushita N, Takami Y, Kimura M, et al. Role of NAD-dependent deacetylases SIRT1 and SIRT2 in radiation and cisplatin-induced cell death in vertebrate cells. Genes Cells 2005;10:321–32.
15. Comi P, Chiaramonte R, Maier JA. Senescence-dependent regulation of type 1 plasminogen activator inhibitor in human vascular endothelial cells. Exp Cell Res 1995;219:304–8.
16. Hayashi T, Matsui-Hirai H, Miyazaki-Akita A, et al. Endothelial cellular senescence is inhibited by nitric oxide: implications in atherosclerosis associated with menopause and diabetes. Proc Natl Acad Sci U S A 2006;103:17018–23.
17. Farb A, Sangiorgi G, Carter AJ, et al. Pathology of acute and chronic coronary stenting in humans. Circulation 1999;99:44–52.
18. Virmani R, Guagliumi G, Farb A, et al. Localized hypersensitivity and late coronary thrombosis secondary to a sirolimus-eluting stent: should we be cautious? Circulation 2004;109:701–5.
19. Honda Y, Fitzgerald PJ. Stent thrombosis: an issue revisited in a changing world. Circulation 2003;108:2–5.
20. Steffel J, Latini RA, Akhmedov A, et al. Rapamycin, but not FK-506, increases endothelial tissue factor expression: implications for drug-eluting stent design. Circulation 2005;112:2002–11.
21. Muldowney JA 3rd, Stringham JR, Levy SE, et al. Antiproliferative agents alter vascular plasminogen activator inhibitor-1 expression: a potential prothrombotic mechanism of drug-eluting stents. Arterioscler Thromb Vasc Biol 2007;27:400–6.
22. Hashimoto A, Miyakoda G, Hirose Y, Mori T. Activation of endothelial nitric oxide synthase by cilostazol via a cAMP/protein kinase A- and phosphatidylinositol 3-kinase/Akt-dependent mechanism. Atherosclerosis 2006;189:350–7.
23. Daemen J, Serruys PW. Drug-eluting stent update 2007: part II: unsettled issues. Circulation 2007;116:316–28.
24. North BJ, Marshall BL, Borra MT, Denu JM, Verdin E. The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. Mol Cell 2003;11:437–44.
25. Milne JC, Lambert PD, Schenk S, et al. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature 2007;450:712–6.
26. Lee SW, Park SW, Kim YH, et al. Drug-eluting stenting followed by cilostazol treatment reduces late restenosis in patients with diabetes mellitus the DECLARE-DIABETES trial (A randomized comparison of triple antiplatelet therapy with dual antiplatelet therapy after drug-eluting stent implantation in diabetic patients). J Am Coll Cardiol 2008;51:1181–7.
27. Tanaka K, Gotoh F, Fukuuchi Y, et al. Effects of a selective inhibitor of cyclic AMP phosphodiesterase on the pial microcirculation in feline cerebral ischemia. Stroke 1989;20:668–73.
28. Kim KY, Shin HK, Choi JM, Hong KW. Inhibition of lipopolysaccharide-induced apoptosis by cilostazol in human umbilical vein endothelial cells. J Pharmacol Exp Ther 2002;300:709–15.
29. Tani T, Uehara K, Sudo T, Marukawa K, Yasuda Y, Kimura Y. Cilostazol, a selective type III phosphodiesterase inhibitor, decreases triglyceride and increases HDL cholesterol levels by increasing lipoprotein lipase activity in rats. Atherosclerosis 2000;152:299–305.

Key Words: sirolimus ▪ everolimus ▪ antiplatelet therapy ▪ endothelial senescence.

Original Article

Validity and Usefulness of Aortic Arch Calcification in Chest X-Ray

Hiroko Hashimoto[1], Katsuya Iijima[1], Masayoshi Hashimoto[2], Bo-Kyung Son[1], Hidetaka Ota[1], Sumito Ogawa[1], Masato Eto[1], Masahiro Akishita[1], and Yasuyoshi Ouchi[1]

[1]Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[2]Department of General Medicine, Division of General Medicine Science, Kobe University Hospital, Hyogo, Japan

Background: Arterial calcification is associated with cardiovascular (CV) disease, to be leading to vessel wall stiffness and causing the management of hemodynamics in the elderly more difficult. Here, we compared the extent of calcification in the aortic arch by reviewing chest X-rays to that in the abdominal aorta as assessed by more detailed examinations. In addition, the validity of the grading and the relationship of this useful grading to clinical risk factors were evaluated.
Methods and Results: The extent of aortic arch calcification (AAC) on a postero-anterior plain chest X-ray was divided into four grades (0 to 3). First, AAC grade was assessed in patients who underwent two quantitative examinations for abdominal aortic calcification; lateral radiograph of lumbar spine and/or computer tomography, and was positively correlated with the abdominal aortic calcification level. Subsequently, AAC grade in 239 out-patients (115 men; mean age, 61.9 years) was also evaluated, and was 0, 1, 2, and 3 in 46%, 22%, 29%, and 4% of the population, respectively, was significantly associated with pulse pressure and intima-media thickness. AAC grade in patients with diabetes or renal dysfunction was significantly higher than in those without each risk, but there was no association with other risk factors. In addition, AAC grade was positively correlated with risk factor clustering.
Conclusion: Assessment of AAC detectable on a chest X-ray is very useful and its grade reflects the magnitude of calcified change in the whole aorta. In addition, AAC evaluation may provide supportive information for atherosclerotic risk stratification.

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Key words; Aortic arch calcification, Grading, Risk factor clustering, Atherosclerotic risk stratification

Introduction

Arterial calcification is a complication of advanced atherosclerosis. Arterial stiffness resulting from calcification is shown to be strongly associated with variable blood pressure (such as isolated systolic hypertension and orthostatic hypotension)[1, 2] and an increase in myocardial afterload, leading to left ventricular hypertrophy[3]. In addition, loss of elastic recoil due to arterial calcification results in unstable hemodynamic consequences, leading to a decline in end-organ perfusion[1]. In general, arterial calcification occurs at two anatomical sites in the arterial wall, the media and the intima[4, 5]. Medial calcification, which is frequently seen in the elderly[6], diabetes[7, 8] and chronic renal failure patients on dialysis[9, 10], is observed as continuous linear deposits along the internal elastic lamina. On the other hand, intimal calcification, which is seen as patchy scattered deposits only occurring within atherosclerotic plaques, has been shown to be associated with plaque vulnerability[11, 12].

Several imaging examinations have been employed to detect and quantify macroscopic arterial calcification. Plain radiographs have traditionally been a valuable and inexpensive tool for detecting arterial calcification, especially aortic calcification, in routine clinical work. Arterial medial calcification is radiographically visible as a radio-opaque finding, like a linear

Address for correspondence: Katsuya Iijima, Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
E-mail: katsu-tky@umin.ac.jp
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