hypercholesterolemia should be determined individually according to their physical activities. It is noted that the elderly are more susceptible to drug-related adverse effects than the younger since renal and liver functions, required for metabolizing drugs, in the elderly are relatively weaker.

Keywords: cardiovascular event, elderly, hypercholesterolemia, Japanese, statin.

Introduction

It is well known that cardiovascular events occur in elderly people more frequently than in the younger population. It is also known that the incidence of these events increases as serum cholesterol levels are elevated. In Japan, populations of elderly people are rapidly increasing and serum cholesterol levels have been clearly rising in all ranges of ages probably due to westernization of our dietary habits.[1] Therefore, a rapid increase in atherosclerotic diseases is anticipated in Japan, especially in the elderly, without appropriate prevention.

Data obtained in many clinical studies performed in Western countries have demonstrated that cholesterol-lowering therapy with HMG-CoA reductase inhibitors, statins, reduces cardiovascular events by 26–37%.[2–4] Therefore, therapeutic intervention to control serum cholesterol levels is widely accepted. So far, guidelines for controlling cholesterol levels have been established in several countries, such as ATPIII (http://www.nhlbi.nih.gov/guidelines/cholesterol/atp_iii.htm) in the USA. Since the incidence of cardiovascular events in the Japanese population is clearly lower than that in Western countries, establishment of the Japanese guideline has been considered necessary. The first Japanese guideline was established by the Japanese Atherosclerosis Society in 1997 and it has been revised in 2002 (*http://jas.umin.ac.jp*). Since the subjects for the guideline are those aged ≤ 65 years, the guideline for elderly Japanese has been expected to be established.

In 1996–99, the research group for 'Establishing Japanese guidelines for treating atherosclerotic diseases in the elderly' was organized as part of the Comprehensive Research on Aging and Health conducted by the Japanese Ministry for Health, Labour and Welfare and the first guideline was proposed in 1999 (Kita & Hata *et al.* unpublished report to the Japanese Ministry of Health and Welfare 1999). In this guideline, the target cholesterol levels for the elderly were recommended to be 20 mg/dL higher than those for the younger population, based on the comparison of relative risk increase in relation to serum cholesterol levels between younger people and the elderly (Kita & Hata *et al.* unpublished report to the Japanese Ministry of Health and Welfare 1999). Since then, several important clinical datasets in Western countries and results of studies conducted in Japan,[2–4] such as the KLIS,[5,6] the J-LIT and PATE have been produced.[7–9] Therefore, the research group was again organized in 1999–2002 in order to conduct a research project entitled 'Long-term prognosis of the elderly with hyperlipidemia' (chaired by T. Kita) as a part of the Comprehensive Research on Aging and Health with a view to re-evaluating the proposed guideline (Kita & Hata *et al.* unpublished report to the Japanese Ministry of Health and Welfare 1999). The research group has concluded that serum cholesterol levels in Japanese aged 65–74 years are recommended to be controlled in the same way as for patients aged ≤ 65 years by following the Guideline for Diagnosis and Treatment of Atherosclerotic Cardiovascular Diseases (2002) by the Japan Atherosclerosis Society (http://jas.umin.ac.jp/), and that for those aged ≥ 75 years the control levels should be determined individually based on their physical activities (Kita & Matsuzawa *et al.* unpublished report to the Japanese Ministry of Health and Welfare 2002).

Clinical data in Western countries

Secondary prevention studies such as 4S and CARE have been analyzed with a focus on the elderly.[10,11] In both studies, treatment with simvastatin and pravastatin in the elderly patients was as safe and effective for reducing serum cholesterol levels as it was in younger patients.[10,11] In the 4S study, 4444 patients with established coronary heart diseases were divided into simvastatin and placebo groups, and followed for 5.4 years.[10] In this study, simvastatin treatment reduced total cholesterol levels by 26% in the elderly aged 65–70 years and by 25% in younger patients,[10] indicating that the cholesterol lowering effect of simvastatin in the elderly is similar to that in the younger. The relative risk reduction of major coronary events, including coronary artery death and non-fatal myocardial infarction, by simvastatin in the elderly patients was 34%, similar to that in younger patients aged < 65 years.[10] In the CARE study, 4159 patients were divided into pravastatin and placebo groups and followed for 5 years.[11] In this study, pravastatin treatment reduced total cholesterol levels by 19% in the elderly aged 65–75 years and by 20% in patients aged < 65 years,[11] indicating that the cholesterol lowering effect of simvastatin in the elderly is similar to that in younger patients. The relative risk reduction in the elderly group was 39% while that in the younger was 13%.[11] Because of the higher absolute risk and greater effect on risk reduction in the elderly group, the number

needed to treat (NNT) in the 5-year follow-up period in the elderly group was 15 while that in the younger group was 67.[11]

Recently, the results of the PROSPER study have been published.[12] In this study, approximately 5800 high-risk patients aged 70–82 (mean 75 years) with normal total cholesterol levels (mean 217 mg/dL) were divided into pravastatin and placebo groups, and followed for 3 years. In this elderly population, the statin reduced coronary events by 19%. Since the preventive effects by statins become obvious in 1–2 years after starting the medication in many studies,[2–4] the risk reduction ratio in the PROSPER study could be relatively smaller during the 3-year follow-up period.[12] In the ASCOT study, approximately 19 000 hypertensive high-risk patients with total cholesterol levels of ≤ 250 mg/dL (213 mg/dL average), aged 40–79 years (mean 63 years), were assigned into placebo and 10 mg/day atorvastatin groups, and followed for 3 years.[13] The results showed that treatment with atorvastatin reduced coronary events by 36%. The risk reduction in the subgroup aged ≥ 60 years was also 36%, which was similar to that in younger patients aged < 60 years. Thus, it has been demonstrated in studies conducted in Western countries that cholesterol-lowering therapy in the elderly brings similar, or even better, effects in the prevention of coronary events, compared with its effects on younger patients.

It has been demonstrated that cholesterol lowering therapy by statins slowed the narrowing of coronary arteries and reduced intima-media thickness in carotid arteries.[14,15] Thus, cholesterol lowering by statins could stabilize atheromatous plaque, thereby inhibiting the event occurrence.

Clinical data in Japan

The Hisayama study was an epidemiological study conducted in the Hisayama community in Japan.[16] In this study, where 2673 people aged ≥ 40 years were followed from 1988 to 1996, the absolute risk for ischemic heart diseases (myocardial infarction and sudden death) was reported to be 2.3/1000/year and that for cerebral infarction to be 3.1/1000/year.[17]

The J-LIT study was a cohort observational study in Japan. In this study, approximately 50 000 hypercholesterolemic patients aged ≤ 70 years undergoing 5–10 mg/day simvastatin treatment were followed for 6 years. A subanalysis focusing on elderly patients without prior coronary events was performed.[18] In both the elderly group, aged 65–70 years (mean 67 years) and consisting of 9860 patients, and the younger group, aged ≤ 64 years (mean 55 years) and consisting of 32 500 patients, total cholesterol levels were approximately 270 mg/dL at enrollment and 210–220 mg/dL during follow-up periods under simvastatin treatment. Changes in low-density lipoprotein (LDL)-cholesterol levels were also similar: levels of approximately 180 mg/dL at the baseline were reduced to approximately 130 mg/dL in the follow-up periods in both groups. No severe drug-related adverse effects occurred in either group. Thus, statin treatment in the elderly is as safe and effective for reducing serum cholesterol levels as it is in younger patients. The doses of the statin were lower than those used in Western countries, where 20–40 mg/day simvastatin was used.[2]

In the J-LIT study, the incidence of coronary events (sudden cardiac death and acute myocardial infarction) in the elderly was 1.30/1000/year and that in the younger 0.8/1000/year. When occurrence of angina was included in coronary events, the incidence in the elderly was 2.25/1000/year and that in the younger 1.35/1000/year. Cox-biohazard analysis revealed that the relative risks of coronary events increased by 1.7% as serum LDL-cholesterol levels increased by 1 mg/dL, which were similar in both groups.[18] Importantly, in any LDL-cholesterol levels, the absolute risk in the elderly was higher than that in the younger. Generally, coronary events occur twice as often in men as in women, which was also observed in the J-LIT study.[7,8] In the J-LIT study, 35% of the study subjects were male in the younger group and 21% were male in the elderly group.[18] Therefore, upon interpretation of this J-LIT data, the male : female ratio should be considered. Indeed, in male patients, the coronary events (sudden cardiac death and acute myocardial infarction) occurred at a rate of 2.45/1000 patients/year in the elderly and 1.41/1000 patients/year in younger patients.

The KLIS study was planned as a primary prevention study for male patients aged 45–74 years with serum cholesterol levels ≥ 220 mg/dL.[5,6] Enrolled patients were assigned into a conventional therapy group and a pravastatin group, and followed for 5 years. However, since the results of several studies revealed superior effects of statin therapy for the event prevention during the study period, the assignment could not be kept completely. As a result, 2219 cases in the pravastatin group and 1634 cases in the conventional therapy group were analyzed. Coronary events (sudden death, myocardial infarction, coronary intervention and bypass surgery) occurred in 5.95/1000 per year in the conventional therapy group and 5.77/1000 per year in the pravastatin group. Cerebral infarction occurred in 5.15/1000 per year in the conventional therapy group and 4.19/1000 per year in the pravastatin group. In the pravastatin group, 1105 cases were of good compliance for the drug-intake. The relative risk of coronary events plus cerebral infarction of the good-compliance group was 0.57 (0.54–0.98) compared with that of the conventional therapy group. A subanalysis examining those aged ≥ 65 years in this study revealed a tendency similar to that observed in the J-LIT study.[18] Namely, coronary events increased as

serum LDL-cholesterol levels increased in both elderly and younger groups, and the absolute risks in the elderly were higher than those in the younger in any given LDL-levels (Sasaki *et al.* in preparation).

In the PATE study, 665 patients (male ratio 21%) aged ≥ 60 years (mean 73 years) with serum total cholesterol levels of 220–280 mg/dL were followed for 3–5 years (mean 3.9 years) under treatment with low-dose (5 mg) or high-dose (10–20 mg) pravastatin.[9] In this study, events were defined as cerebral bleeding, cerebral infarction, transient ischemic attack, subarachnoid hemorrhage, myocardial infarction, angina pectoris, cardiac failure, arrhythmia, arteriosclerosis obliterance, dissecting aortic aneurysm, and peripheral artery thrombosis. During the follow-up period, acute myocardial infarction occurred in 11 cases (4.2/1000/year). In the patient group without diabetes and with serum cholesterol levels of < 253 mg/dL and triglyceride levels of ≥ 133 mg/dL, the event-free ratio in the high-dose group was significantly higher than that in the low-dose group.

Thus, we could expect similar, or even more beneficial, effects of cholesterol-lowering therapy to reduce cardiovascular events in elderly Japanese compared with those in the younger population, although the studies described above appear to be somewhat indirect. Urgently and absolutely required are complete epidemiological and/or interventional large-scale studies, from which we can definitely estimate the absolute risks and the risk reduction rates in the current Japanese population.

Cerebral infarction and hypercholesterolemia

Cerebral infarction is also a disease that occurs more frequently in the elderly. Cerebral infarction is classified into following three: (i) lacunar infarction caused by small artery occlusion which is correlated with hypertension; (ii) cardiogenic cerebral embolism, which is usually associated with atrial fibrillation; and (iii) cerebral infarction caused by atherothrombotic arterial occlusion. Hypercholesterolemia is considered to be linked to atherothrombotic occlusion.

In the 4S secondary prevention study, simvastatin reduced total strokes by 35%.[2] The data obtained in secondary prevention studies with pravastatin, including the LIPID and CARE studies, have been combined and analyzed.[19] The results demonstrated that pravastatin treatment reduced total strokes by 22% and nonhemorrhagic strokes by 23%.[19] In the ASCOT study, atorvastatin reduced fatal and non-fatal strokes by 27%.[13] In the MRC/BHF Heart Protection Study, where approximately 20 000 high-risk patients aged 40–80 years had been randomly assigned into placebo and simvastatin-treated groups and followed for 5 years, simvastatin reduced ischemic strokes by 29%.[20] In the KLIS study conducted in Japan, the incidence of cerebral infarction was 5.15/1000 per year in the conventional therapy group and 4.19/1000 per year in the pravastatin group.[5,6] In the KLIS study, the incidence of cerebral infarction increased as LDL-cholesterol levels increased in elderly aged ≥ 65 years (Sasaki *et al.* in preparation). In the J-LIT study, the incidence of ischemic cerebrovascular events was 1.41/1000 per year in the subgroup without prior coronary or cerebral infarction (Nakaya *et al.* unpublished data). In both studies, the incidence of ischemic cerebral events was clearly higher in the elderly than that in the younger. Thus, evidence is accumulating to support the preventive effects of serum cholesterol-lowering on the occurrence of cerebral infarction. We may expect risk reduction for not only coronary events but also cerebral infarction in cholesterol-lowering therapy. Although the incidence of coronary events in Japan is much lower compared with that in Western countries, the incidence of cerebrovascular events are similar. Since incidence of cerebrovascular events in Japan is similar to that of coronary events, impact of the prevention of cerebrovascular events is as large as that of coronary events in Japan.

Conclusions: Strategy for treating elderly Japanese with hypercholesterolemia

As reviewed above, the control of serum cholesterol levels appears effective in risk reduction of cardiovascular events in elderly Japanese as well as in the younger population. The incidence of such events in the elderly is generally higher than that in younger people. Therefore, the elderly would be even more suitable subjects for preventative intervention. Although it may take long periods to develop atherosclerosis, the preventive effects for cardiovascular events become apparent in 1–2 years after cholesterol-lowering therapy has started, as demonstrated in many studies.[2,3,11,12,20] Therefore, it is not too late for us to start cholesterol-lowering therapy in the elderly. We have concluded after discussion in the research group 'Long-term prognosis of elderly Japanese with hypercholesterolemia' that we could expand the subjects of the Guideline for Diagnosis and Treatment of Atherosclerotic Cardiovascular Diseases by the Japan Atherosclerosis Society (2002) to include elderly Japanese aged ≤ 74 years (Kita & Matsuzawa *et al.* unpublished report to the Japanese Ministry of Health and Welfare 2002). In the guideline, patients are divided into several categories based on risk factors and the target cholesterol levels for each category is indicated (http://jas.umin.ac.jp/). Aging, ≥ 45 years for men and ≥ 55 years for women, is defined as a risk factor. Therefore, the target total cholesterol level for the elderly aged 65–74 years without additional risk factors is to be less

than 220 mg/dL and the target LDL-cholesterol level, less than 140 mg/dL. The target levels become lower when elderly patients possess additional risk factors. As described in the guideline (http://jas.umin.ac.jp/), the control of cholesterol levels should be started by changing life styles, followed by drug therapy when appropriate cholesterol levels are not obtained.

For the elderly aged ≥ 75 years, few data for Japanese are available at the moment. Furthermore, it was reported that all causes of mortality increased in the group with lower total cholesterol levels due to an increase in death from infections and malignant tumors in an investigation in Holland, where people aged ≥ 85 years were enrolled.[21] Furthermore, in the Honolulu Heart Program, Japanese–Americans aged 75–93 years (mean 78 years) with a mean total cholesterol level of 149 mg/dL have been reported to have higher mortality than the other groups with the levels at 178, 199 and 232 mg/dL.[22] The physical and nutritional conditions of the highly-aged elderly are various and low cholesterol levels may reflect their worsened health conditions. Therefore, we concluded that, for the highly-aged elderly ≥ 75 years, the target cholesterol levels should be determined individually according to physical and nutritional factors, although a higher absolute risk of cardiovascular events would be expected in the elderly aged ≥ 75 years.

Finally, we again emphasize that physicians should be more careful in their use of drugs in elderly patients since physiological functions of the elderly, such as renal and liver functions required for metabolizing drugs, are not as good as those of the younger patients.

The recommended strategy for treatment for elderly Japanese with hypercholesterolemia

Patients aged 65–74 years

Follow the Guideline for Diagnosis and Treatment of Atherosclerotic Cardiovascular Diseases by the Japan Atherosclerosis Society (2002) (http://jas.umin.ac.jp/).

Patients aged ≥ 75 years

The target values of total and LDL-cholesterol levels should be determined individually.

Points of consideration for treatment of elderly with hypercholesterolemia

1 Cholesterol-lowering therapy reduces relative risk of coronary events in not only the younger but also in the elderly to a similar extent.
2 The elderly would be even more suitable subjects of lipid-lowering therapy, since the absolute risk in the elderly is higher than that in the younger.
3 The elderly might be more susceptive to drug-related adverse effects than the younger since renal and liver functions, required for metabolizing drugs, in the elderly are weaker.

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References

1 Kuzuya M, Ando F, Iguchi A, Shimokata H. Changes in serum lipid levels during a 10-year period in a large Japanese population. A cross-sectional and longitudinal study. *Atherosclerosis* 2002; **163**: 313–320.
2 The Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; **344**: 1383–1389.
3 Shepherd J, Cobbe SM, Ford I *et al.* Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N Engl J Med* 1995; **333**: 1301–1307.
4 Sacks FM, Tonkin AM, Shepherd J *et al.* Effect of pravastatin on coronary disease events in subgroups defined by coronary risk factors: the Prospective Pravastatin Pooling Project. *Circulation* 2000; **102**: 1893–1900.
5 The Kyushu Lipid Intervention Study Group. Pravastatin use and risk of coronary events and cerebral infarction in Japanese men with moderate hypercholesterolemia: the Kyushu Lipid Intervention Study. *J Atheroscler Thromb* 2000; **7**: 110–121.
6 Sasaki J, Arakawa K, Iwashita M, Matsushita Y, Kono S. Reduction in serum total cholesterol and risks of coronary events and cerebral infarction in Japanese men: the Kyushu Lipid Intervention Study. *Circ J* 2003; **67**: 473–478.
7 Matsuzaki M, Kita T, Mabuchi H *et al.* Large scale cohort study of the relationship between serum cholesterol concentration and coronary events with low-dose simvastatin therapy in Japanese patients with hypercholesterolemia. *Circ J* 2002; **66**: 1087–1095.
8 Mabuchi H, Kita T, Matsuzaki M *et al.* Large scale cohort study of the relationship between serum cholesterol concentration and coronary events with low-dose simvastatin therapy in Japanese patients with hypercholesterolemia and coronary heart disease: secondary prevention cohort study of the Japan Lipid Intervention Trial (J-LIT). *Circ J* 2002; **66**: 1096–1100.
9 Ito H, Ouchi Y, Ohashi Y *et al.* A comparison of low versus standard dose pravastatin therapy for the prevention of cardiovascular events in the elderly: the pravastatin antiatherosclerosis trial in the elderly (PATE). *J Atheroscler Thromb* 2001; **8**: 33–44.
10 Miettinen TA, Pyorala K, Olsson AG *et al.* Cholesterol-lowering therapy in women and elderly patients with myocardial infarction or angina pectoris: findings from the Scandinavian Simvastatin Survival Study (4S). *Circulation* 1997; **96**: 4211–4218.
11 Lewis SJ, Moye LA, Sacks FM *et al.* Effect of pravastatin on cardiovascular events in older patients with myocardial

infarction and cholesterol levels in the average range. Results of the Cholesterol and Recurrent Events (CARE) trial. *Ann Intern Med* 1998; **129**: 681–689.
12 Shepherd J, Blauw GJ, Murphy MB *et al.* Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. *Lancet* 2002; **360**: 1623–1630.
13 Sever PS, Dahlof B, Poulter NR *et al.* ASCOT investigators. Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial. *Lancet* 2002; **361**: 1149–1158.
14 Teo KK, Burton JR, Buller CE *et al.* Long-term effects of cholesterol lowering and angiotensin-converting enzyme inhibition on coronary atherosclerosis: The Simvastatin/ Enalapril Coronary Atherosclerosis Trial (SCAT). *Circulation* 2000; **102**: 1748–1754.
15 Smilde TJ, van Wissen S, Wollersheim H, Trip MD, Kastelein JJ, Stalenhoef AF. Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolaemia (ASAP): a prospective, randomised, double-blind trial. *Lancet* 2001; **357**: 577–581.
16 Tanizaki Y, Kiyohara Y, Kato I *et al.* Incidence and risk factors for subtypes of cerebral infarction in a general population: the Hisayama study. *Stroke* 2000; **31**: 2616–2622.
17 Fujishima M. [Cardiovascular disease in the elderly: the Hisayama Study.] *Nippon Ronen Igakkai Zasshi* 1999; **36**: 16–21. (In Japanese.)
18 Horiuchi H, Kita T, Mabuchi H *et al.* Primary cardiovascular events and serum lipid levels in elderly Japanese with hyperchlesterolemia under 6-year simvastatin treatment: A sub analysis of the J-LIT study. *J Am Geriat Soc* 2004 (in press).
19 Byington RP, Davis BR, Plehn JF *et al.* Reduction of stroke events with pravastatin: the Prospective Pravastatin Pooling (PPP) Project. *Circulation* 2001; **103**: 387–392.
20 Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002; **360**: 7–22.
21 Weverling-Rijnsburger AW, Blauw GJ, Lagaay AM, Knook DL, Meinders AE, Westendorp RG. Total cholesterol and risk of mortality in the oldest old. *Lancet* 1997; **350**: 1119–1123.
22 Schatz IJ, Masaki K, Yano K, Chen R, Rodriguez BL, Curb JD. Cholesterol and all-cause mortality in elderly people from the Honolulu Heart Program: a cohort study. *Lancet* 2001; **358**: 351–355.

RELATION BETWEEN RETIREMENT AND SUBSEQUENT HEALTH STATUS IN HIGHLY EDUCATED OLDER MEN

To the Editor: Retirement from work brings a great change in mental, physical, and social activities in life. Although it has often been conjectured that sedentary life styles after retirement may adversely affect physical and mental health and accelerate aging,[1–3] the relation between retirement age and subsequent health status remains uncertain. The aim of this study was to examine the relationship between retirement age and susceptibility to subsequent medical illnesses or death in elderly male subjects with higher levels of education in Japan.

The survey was conducted between April 2003 and June 2003 using a structured 13-item questionnaire composed of detailed questions about retirement age (or working status if still employed), health status, cognitive function,[3] daily physical activities,[4] spousal health status, and participation in planned exercises such as walking, jogging, bicycling, sports, social activities such as

housework, gardening, hobbies, and social volunteer activities more than twice per week. The questionnaire was mailed to male subjects aged 65 and older who were randomly selected from a graduation list of the School of Engineering, Tohoku University. Subjects were excluded for analysis if they had any significant history of medical, surgical, or psychiatric illness or had any symptoms or signs of cerebrovascular or neurological disease or psychiatric illness at the age of 60 when retirement usually becomes an option or the main reason for their retirement was serious health problems such as cardiac disease, respiratory disease, cerebrovascular disease, or cancers. If a subject had died before this survey, his spouse was asked to answer the questions. Written informed consent was obtained, and the local institutional review board approved the study protocol. Chi-square test or one-way analysis of variance was used to detect statistical significance between groups. Cox proportional hazards regression analysis was used to evaluate the risk of morbidity or mortality according to retirement age. All multivariate models reported include the covariates in the questionnaire.

Of 936 selected subjects, 642 (68.6%) responded, and 31 were excluded due to the exclusion criteria, leaving 611 (492 retirees and 119 nonretirees) for analysis. The current age of all participants ranged between 72 and 88, with a mean ± standard deviation of 79.5 ± 6.3. The study cohort was divided into four groups according to retirement age (those who retired from work at aged 50–59

Table 1. Characteristics and Risk of Morbidity and Mortality in Retired and Nonretired Older Men

Characteristic	Retirement Age: 50–59 (Group A; n = 56)	60–69 (Group B; n = 294)	70–79 (Group C; n = 142)	Nonretirees (Group D; n = 119)	*P*-value†
Age, mean ± SD	79.2 ± 2.6	79.4 ± 2.4	79.8 ± 2.1	79.6 ± 2.2	.24
Follow-up period after retirement, years, mean ± SD	19.6 ± 2.7	12.7 ± 3.6	5.1 ± 2.8	0	
Cognitive function, n (%)					
With dementia	5 (8.9)	21 (7.1)	10 (7.0)	1 (0.8)	.06
Without dementia	51 (91.1)	273 (92.9)	132 (93.0)	118 (99.2)	
P-value*		.58	.77	.01	
Activities of daily living, n (%)					
Dependent	8 (14.3)	33 (11.2)	21 (14.8)	2 (1.7)	.003
Independent	48 (85.7)	261 (88.8)	121 (85.2)	117 (98.3)	
P-value*		.20	1.00	.01	
Exercises, n (%)					
Participant	17 (30.4)	148 (50.3)	64 (45.1)	48 (40.3)	.04
Non-participant	39 (69.6)	146 (49.7)	78 (54.9)	71 (59.7)	
P-value*		.01	.08	.24	
Social activities, n (%)					
Present	14 (25.0)	69 (23.5)	30 (21.1)	22 (18.5)	.67
Absent	42 (75.0)	225 (76.5)	112 (78.9)	97 (81.5)	
P-value*		.86	.57	.32	
Volunteer activities, n (%)					
Participant	9 (16.1)	50 (17.0)	17 (12.0)	17 (14.3)	.57
Non-participant	47 (83.9)	244 (83.0)	125 (88.0)	102 (85.7)	
P-value*		1.00	.49	.82	
Spousal health status, n (%)					
Absent	7 (12.5)	38 (12.9)	11 (7.7)	15 (12.6)	.81
Healthy	46 (82.1)	241 (82.0)	125 (88.1)	98 (82.4)	
Disability	3 (5.4)	15 (5.1)	6 (4.2)	6 (5.0)	
P-value*		.99	.53	1.00	
Health status, n (%)					
Healthy	30 (53.5)	162 (55.1)	89 (62.7)	99 (83.2)	<.001
Medical illnesses	16 (28.6)	102 (34.7)	43 (30.3)	20 (16.8)	
Death	10 (17.9)	30 (10.2)	10 (7.0)	0 (0.0)	
P-value*		.23	.07	<.001	
Adjusted hazard ratio of medical illnesses or death (95% confidence interval)	1.00	0.75 (0.47–1.19)	0.50 (0.29–0.84)	0.26 (0.13–0.52)	
P-value*		.22	.009	<.001	

*P-values of individual characteristics were calculated based upon a comparison with retired men aged 50 to 59.
†P-values were calculated using one-way analysis of variance or chi-square test between four groups and variables.

(Group A, n = 56), 60–69 (Group B, n = 294), and 70–79 (Group C, n = 142)) and those who continued to work (Group D, n = 119), and the risk of medical illnesses or death according to these groups was determined. No significant differences were found at the age of 60 between the four groups in vascular risk factors, including hypertension, diabetes mellitus, hyperlipidemia and cigarette smoking, medications prescribed, or dietary treatment.

Table 1 summarizes demographic characteristics of the four groups, including age, and possible confounding factors for medical illnesses. Group D was significantly more independent in activities of daily living than the other groups ($P = .003$). Over a median follow-up period of 9.1 years (range 0–27 years) after retirement, medical illnesses without death (171 cases) or those with death (60 cases) occurred in 231 cases (26, 132, 53, and 20 cases in group A, B, C, and D, respectively). Group C and Group D had significantly lower risk of medical illnesses or death than Group A after adjustment for other confounding factors (hazard ratio (HR) = 0.50, 95% confidence interval (CI) = 0.29–0.84, $P = .009$; HR = 0.26, 95% CI = 0.13–0.52, $P > .001$, respectively) (Table 1). Of the principal medical illnesses reported in all participants, cardiac disease was the most common (63), followed by cancer (32), cerebrovascular disease (29), respiratory disease (21), orthopedic disease (15), and other (71), which is in accordance with the normal elderly population.

This retrospective study demonstrates a significant negative association between retirement age and less medical illness or death in elderly subjects with higher levels of education. Because many types of employment require considerable physical activity, retirees who follow a sedentary life for a longer time may place themselves at increased risk of cardiovascular and cerebrovascular disease and cancer. Work-associated physical activity generally decreases after retirement, which is seldom compensated by planned participation in physical activities after retirement.[1–3] These results indicate that, even after reaching retirement age, elderly subjects who are willing to work should continue to work as long as possible to sustain better overall health status.

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REFERENCES

1. Rogers RL, Meyer JS, Mortel KF. After reaching retirement age physical activity sustains cerebral perfusion and cognition. J Am Geriatr Soc 1990;38:123–128.
2. Paffenbarger RS Jr, Hyde RT, Wing AL et al. The association of changes in physical-activity level and other lifestyle characteristics with mortality among men. N Engl J Med 1993;328:538–545.
3. Verghese J, Lipton RB, Katz MJ et al. Leisure activities and the risk of dementia in the elderly. N Engl J Med 2003;348:2508–2516.
4. Katz S, Downs TD, Cash HR et al. Progress in development of the index of ADL. Gerontologist 1970;10:20–30.

特集
高齢者一般外来に有用な老年病診断学の知識(2)
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初診外来 どこまでみるか，専門外来への紹介の岐路

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KEY WORD
高齢者医療
チーム医療
初診外来

POINT
- 高齢者医療と臓器別専門家がチーム医療を形成する．
- 高齢患者の初診外来はフローチャートが重要である．
- 専門外来では寝たきり，痴呆症の診察は困難である．
- 紹介の条件の1つはQOLの向上である．
- 多臓器障害は高齢者専門外来が望ましい．

0387-1088/04/¥500/論文/JCLS

はじめに

高齢者の初診外来では短時間で多くの情報を入手し，検査を行い，判断する必要がある．初診外来の目的は病気の診断と治療であるが，入院の必要性の有無の判断が最大の重要なポイントであり，治療方針の判断を短時間で行う必要性がある．初診だからといって病気を見逃すわけにはいかない．

専門外来との関係は院内であらかじめ検討しておく必要がある．胃カメラ，気管支鏡，マルクなどを専門家に依頼することもあるし，患者や介護者が他科受診を希望することもあるが，始めから専門医療を必要とする場合もあるし，紹介する必要がある場合もある．高齢患者を診るのに最も適切な科が診ればいいわけであるが，その判断が難しい場合もある．すなわち高齢者医療と臓器別専門医療はお互いに補いあい，チーム医療を実践することが重要である．

本稿ではその判断について，われわれの基準を参考として示すこととする．

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初診外来でどこまでみるか

初診ではまず，主訴や検査所見から優先的に対応する病気を決定する必要がある．初診で診断から治療が終了し，1回の受診で治療が完結する場合がある．たとえば膀胱炎，風邪など感染症で軽微な場合は1回の投薬で診療が終了する場合が多い．しかしほかに初診で入院の判断を行う場合や，週に1回程度再診を継続する場合，またはただちに専門外来へ紹介する場合もある．すなわちケースバイケースであるが，高齢者は受診後，病状が悪化する場合もあるし，初診では1回の受診で診断が困難な場合もある．

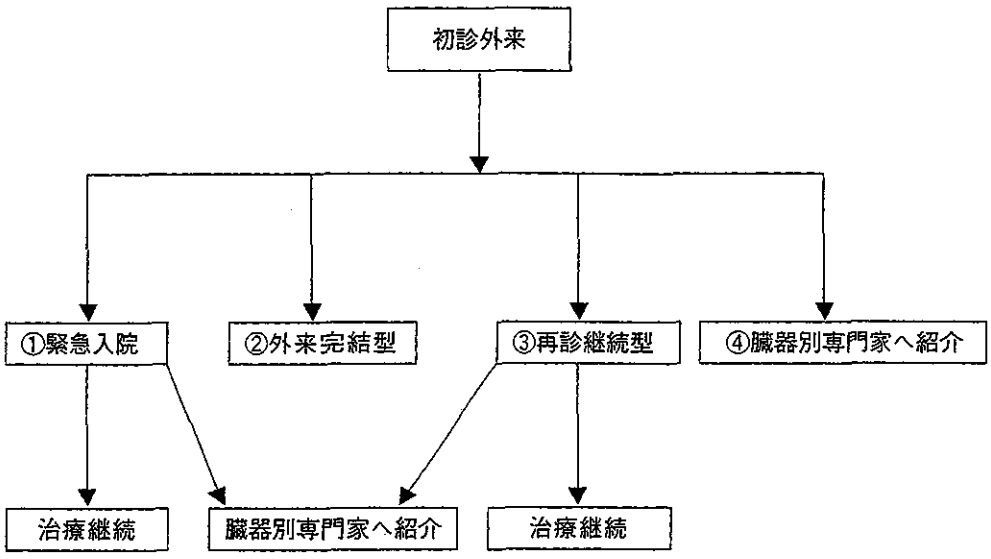

図1　初診外来のフローチャート

つまり外来で多くの場合は医師が短時間に多くの責任ある判断をしているといえよう．図1に初診外来のフローチャートを示す．初診時に決定的なことは緊急入院が必要かどうかの判断であるし，老年科医として責任ある判断ができるかどうかである．病気が重症であり，専門的な検査，診断が必要な場合には他科へ紹介するべきであり，その判断が適切な時期に行われることが重要である．つまり初診であっても必要があれば患者を専門外来へ紹介する必要があるからである．

高齢者総合外来では高齢患者に対して基本的に総合機能評価(CGA)を行う．これにより疾患の診断・治療にとどまらず，より高齢者を深く，全人的に理解するためにADL，IADL，抑うつ，認定機能，QOLを評価することが求められる．高齢患者での慢性疾患の合併率を男女別，年齢別に調べたところ，女性のほうが，男性より合併率が高く，高齢になるほど慢性疾患合併率が上昇することが明らかになっている[1]，つまり高齢患者では単一の疾患を抱えているというより，加齢がリスクとなり，総合的，包括的な医療が求められているといえよう．また表1に高齢者自身が高齢者医療に求める優先順位を示す．これからいえることは有効な医療よりも，障害の改善と予防，QOLの向上に重点をおくことが重要であるという点である．

表1　高齢者自身が高齢者医療に求める優先順位

1位．障害の改善・予防
2位．QOLの向上
3位．介護負担の軽減
4位．精神的健康
5位．高い活動性
6位．有効な医療
7位．老人ホーム入所の回避
8位．問題解決
9位．消費者・利用者の満足
10位．質の高いターミナルケア

一方，急性期病院の入院期間が17日以下さらには14日以内に短期になっていく中で，外来の位置づけ，意義も変わっていく．より外来が重視される形で病院機能は変化していくものと思われる．たとえば外来通院のみで化学療法などの専門的治療を含め，行う場合も多くなってきている．また外来手術のように，入院という方法をとらずに在宅を継続しながらの治療が増加してきている．それゆえにいろいろな意味で，今後も外来の位置づけが変わると考えられる．

専門外来への紹介

高齢者外来では加齢と病気をベースに医療上の判断のみならず，全人的医療をめざし，検査・

表2　専門外来へ紹介する条件

1．65歳未満である場合．
2．高齢患者で単一の疾患があり，特殊で専門的な検査や治療を必要とする場合．
3．寝たきりや痴呆症がなく，治療に際して臓器別専門家が適していると判断される場合．
4．手術，透析などの特殊な治療を必要とする場合．
5．眼科や耳鼻科など専門的なアプローチが優先される場合．

診療以外に高齢者総合機能評価(CGA)を実施し，チーム医療を実践することが重要である．つまり高齢患者はすべて初診で総合的スクリーニングを行うことが望ましい．しかし，もともと臓器別専門家への紹介状がある場合はこの限りでない．また最初から65歳未満であり，病気が単一であり特定できる場合には，専門外来を受診するほうがベターである．臓器別専門外来では特殊，専門的検査に基づく診断と治療を行う．しかし寝たきり，痴呆症などがある場合，多臓器障害がある場合には，また明らかに85歳以上など「超高齢患者」の場合には専門外来より高齢者外来にコンサルテーションの依頼がある場合が多い．

専門外来との関係はあらかじめ検討しておく必要がある．胃カメラ，気管支鏡，マルクなどの専門的な検査が必要な場合には専門家に依頼することもあるし，患者や介護者が他科受診を希望することもあるが，始めから専門医療を必要とする場合もあるし，途中で検査や診断が変わっていくなかで，治療対象の優先順位が変わる場合もあり，途中から紹介する必要がある場合もある．また日常臨床では逆に臓器別専門家が老年科医に対して「超高齢者」や「コミュニケーションがとれないこと」を理由に紹介したり，介護サービスの紹介や主治医意見書の記入を依頼される場合もある．つまり高齢者専門外来と臓器別専門外来はお互いに補完しつつ，協力して高齢者医療にあたるべきである．しかし多くの医師は主治医となると自分で最後まで診たいという気持ちが強いと思われる．そこで専門家に患者を紹介するタイミングが重要となる．紹介すべきときには決断することが重要である．高齢者に関係するチーム医療のメンバーという意識が専門家には少ないかもしれない．

では専門外来への紹介の岐路はどこにあるのだろう．それは対象疾患や医師の能力によっても基準は異なるものと思う．**表2**に専門外来へ紹介する判断基準を示した．これは原則であり，より判断に困るケースも多い．また場合によっては外来を「たらいまわし」にされたり，各科においても押しつけあう場合もある．

それでは，高齢者医療の専門性はどこにあるのだろう．1つは高齢者における多臓器障害の診療にあることはいうまでもない[2]．またせん妄などの精神症状が顕著な場合，高齢で寝たきりである場合，痴呆症を合併しており一般病棟で対応が困難な場合には老年科専門医の出番であろう．また年齢で高齢者医療を規定はできないが，しいていえば平均年齢を超した80歳以上を老年科医が診察することも異論はないであろう．さらに高齢者で退院困難事例においても，老年科医にアドバンテージがあることは誰しも認めるところである．老年科医は介護保険の利用を勧め，介護サービス，介護施設との連携，ショートステイの利用などによる退院支援に優れている．

さらにいえばチーム医療の構築が大学病院であれ，一般病院であれ，高齢者を診ていくうえで最も重要なことである．老年科医と臓器別専門家との連携がとれることが重要であり，アメリカの大学病院などでは老年科医がコンサルテーションに重点をおいている場合も多い．すなわち他科との連携がとれるかが重要なポイントである．一部の大学病院では患者の取り合いになることを恐れ，老年科医との競合をさけ，増設を認めていない大学もある．これなどは超高齢社会において臓器別専門家が老人を単に診ているだけであることを錯覚しており，患者にとり不幸なことである．連携こそが真に患者サー

ビスであることを専門家が理解すべきである．

おわりに

高齢者医療の環境や条件は地域，病院により大きく異なる．一般病院に１人の老年科専門医がいるだけで随分，高齢者医療の概念が他科の医師，看護師においても変わるものと思われる．今後専門外来と高齢者外来が協力して，より良い医療サービスが提供されることを期待している．

文　献

1) 高橋龍太郎：総合的機能評価とは？ 高齢者を知る事典，pp37-39，介護予防研究会編，厚生科学研究所，東京，2000.
2) 荒木　厚：老年病の臓器相関(複合性疾患)．老年病テキスト，pp29-31，メディカルビュー，東京，2002.

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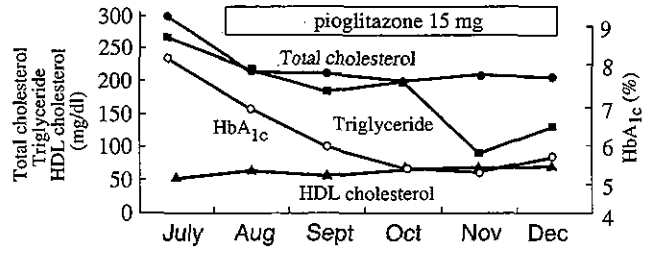

Fasting serum insulin (μU/ml)	45.5	13.0
Insulin sensitivity index	19.5	24.9
Visceral fat area (cm^2)	111.6	104.3
Subcutaneous fat area (cm^2)	162.5	218.5

Fig. 1 Metabolic parameters and abdominal fat areas before and during pioglitazone treatment. HDL = high-density lipoprotein; Hb = hemoglobin.

METABOLIC IMPROVEMENT AND ABDOMINAL FAT REDISTRIBUTION IN WERNER SYNDROME BY PIOGLITAZONE

To the Editor: Werner syndrome is a rare autosomal recessive disorder known for its premature aging phenotype including loss of hair, cataracts, atrophy of peripheral soft tissue, diabetes mellitus, and atherosclerosis. Mutations in the deoxyribonucleic acid (DNA) helicase gene have been identified as the cause of this disease.[1] One common feature of Werner syndrome is insulin resistance, but the mechanism by which insulin resistance occurs in this syndrome is unknown. We have previously described that visceral fat accumulation is strongly associated with insulin resistance in Werner syndrome.[2] We report a case of Werner syndrome in which administration of pioglitazone, a thiazolidinedione derivative, improved insulin sensitivity, glucose tolerance, lipid metabolism, and abdominal fat distribution.

A 46-year old woman with Werner syndrome came to our hospital for glycemic control. After obtaining written informed consent, we analyzed genomic DNA from peripheral leukocytes, which revealed that the patient was homozygote for type 4 mutation in the Werner helicase gene.[3] She was thin (body mass index = 16.5 kg/m^2) but had accumulated visceral fat in excess, as determined using a computed tomography scan at the umbilical level (visceral fat area = 111.6 cm^2, normal range for Japanese women <90).[4] She also had type IIb hyperlipidemia according to World Health Organization classification. She had significant insulin resistance, as determined using an insulin sensitivity index calculated from the value of steady state plasma glucose (19.4, normal range 55–162).[5] After 1 week of treatment with diet, pioglitazone 15 mg daily was initiated. After 16 weeks of pioglitazone treatment, the patient's fasting plasma glucose had decreased from 198 mg/dL to 115 mg/dL, glycated hemoglobin A1c from 8.4% to 5.9% (normal = 5.9% or less), serum total cholesterol from 270 mg/dL to 209 mg/dL (normal = 130–220 mg/dL), serum triglyceride from 301 mg/dL to 90 mg/dL (normal = 80–150 mg/dL), and serum high-density lipoprotein-cholesterol increased from 52 mg/dL to 64 mg/dL (normal ≥ 40 mg/dL). Fasting serum insulin decreased from 45.5 μU/mL to 13.0 μU/mL (normal = 6–26 μU/mL), and insulin sensitivity index had improved to 24.9 (Figure 1, July to November). Although the patient gained weight, from 35.9 kg to 39.0 kg, during the period, her visceral fat area (V) decreased to 104.3 cm^2. In contrast, abdominal subcutaneous fat area (S) increased from 162.5 cm^2 to 218.5 cm^2. As a result, her V/S ratio decreased from 0.69 to 0.48 (normal range for Japanese

<0.4).[4] Liver function monitored using serum transaminase level did not show abnormality throughout the period.

These results suggest that pioglitazone was effective in ameliorating impaired insulin sensitivity, glycemic control, and hyperlipidemia in the patient. Human and animal studies have shown that a possible mechanism for thiazolidinedione to improve insulin sensitivity is through the specific promotion of subcutaneous adipocyte differentiation through the activation of peroxisome proliferator-activated receptor-γ.[6] It has also been reported that troglitazone-treatment of type 2 diabetic patients resulted in subcutaneous fat increase in accordance with improvement of glucose tolerance.[7] It was also proven experimentally that, in lipoatrophic diabetes mellitus, lack of fat is directly associated with insulin resistance and hyperglycemia.[8] Marked atrophy of soft tissues in the extremities, a characteristic feature of Werner syndrome, may at least in part account for the insulin resistance. Leptin administration was recently reported to ameliorate severe insulin resistance in leptin-deficient lipodystrophic patients,[9] but in our patient, serum leptin levels were in the normal range before and during the pioglitazone treatment (data not shown). Therefore, in this case, induction of subcutaneous fat using pioglitazone would have accompanied production of another mediator than leptin to improve insulin sensitivity.

Recently, accumulating evidence suggests that thiazolidinedione has direct antiatherosclerotic effects on vascular cells.[10] Because atherosclerotic vascular disease is a leading cause of middle-age mortality in Werner syndrome, pioglitazone may provide an ideal choice for the treatment of metabolic disorders to improve prognosis of this syndrome.

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REFERENCES

1. Yu CE, Oshima J, Fu YH et al. Positional cloning of the Werner's syndrome gene. Science 1996;272:258–262.
2. Mori S, Murano S, Yokote K et al. Enhanced intra-abdominal visceral fat accumulation in patients with Werner's syndrome. Int J Obes Relat Metab Disord 2001;25:292–295.
3. Matsumoto T, Imamura O, Yamabe Y et al. Mutation and haplotype analyses of the Werner's syndrome gene based on its genomic structure: Genetic epidemiology in the Japanese population. Hum Genet 1997;100:123–130.
4. Yamashita S, Nakamura T, Shimomura I et al. Insulin resistance and body fat distribution. Diabetes Care 1996;19:287–291.
5. Harano Y, Kageyama A, Hirose J et al. Improvement of insulin sensitivity for glucose metabolism with the long-acting Ca-channel blocker amlodipine in essential hypertensive subjects. Metabolism 1995;44:315–319.
6. Adams M, Montague CT, Prins JB et al. Activators of peroxisome proliferator-activated receptor gamma have depot-specific effects on human preadipocyte differentiation. J Clin Invest 1997;100:3149–3153.
7. Mori Y, Murakawa Y, Okada K et al. Effect of troglitazone on body fat distribution in type 2 diabetic patients. Diabetes Care 1999;22:908–912.
8. Gavrilova O, Marcus-Samuels B, Graham D et al. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. J Clin Invest 2000; 105:271–278.
9. Oral EA, Simha V, Ruiz E et al. Leptin-replacement therapy for lipodystrophy. N Engl J Med 2002;346:570–578.
10. Dandona P, Alijada A. A rational approach to pathogenesis and treatment of type 2 diabetes mellitus, insulin resistance, inflammation, and atherosclerosis. Am J Cardiol 2002;90:27G–33G.

High Glucose-Induced Upregulation of Osteopontin Is Mediated via Rho/Rho Kinase Pathway in Cultured Rat Aortic Smooth Muscle Cells

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Objective—Osteopontin is upregulated in the diabetic vascular wall and in vascular smooth muscle cells cultured under high glucose concentration. In the present study, we analyzed the mechanism of high glucose-induced upregulation of osteopontin in cultured rat aortic smooth muscle cells.

Methods and Results—We found that an inhibitor of Rho-associated protein kinase, Y-27632, suppressed osteopontin mRNA expression under high glucose concentration. Transfection of cells with a constitutive active Rho mutant, pSRα-myc-RhoDA, enhanced osteopontin mRNA expression. Furthermore, incubation of cells under high glucose concentration activated Rho, indicating that Rho/Rho kinase pathway mediates high-glucose–stimulated osteopontin expression. Treatment of cells with an inhibitor of protein kinase C, GF109203X, and azaserine, an inhibitor of the hexosamine pathway, suppressed high glucose-induced Rho activation. Glucosamine treatment was shown to activate Rho. Treatment of cells with an inhibitor of MEK1, PD98059, suppressed osteopontin mRNA expression under high glucose concentration. Incubation of cells under high glucose concentration activated ERK. Finally, transfection of cells with pSRα-myc-RhoDA also activated ERK.

Conclusions—In conclusion, our present findings support a notion that Rho/Rho kinase pathway functions downstream of protein kinase C and the hexosamine pathways and upstream of ERK in mediating high-glucose–induced upregulation of osteopontin expression. (***Arterioscler Thromb Vasc Biol.* 2004;24:276-281.**)

Key Words: osteopontin ■ Rho ■ glucose ■ atherosclerosis ■ smooth muscle cells

Osteopontin (OPN)[1] is a multifunctional phosphoprotein secreted by many cell types such as osteoclasts, lymphocytes, macrophages, epithelial cells, and vascular smooth muscle cells (SMC).[1,2] Overexpression of OPN has been found in several physiological and pathological conditions, including immunologic disorders,[3] neoplastic transformation,[4] progression of metastasis,[5] formation of urinary stones,[6] and wound healing.[7]

It was reported that OPN protein and mRNA were expressed in the neointima and in calcified atheromatous plaque.[8] A neutralizing antibody against OPN was found to inhibit rat carotid neointimal formation after endothelial denudation.[9] These results have suggested that OPN promotes the development of atherosclerosis. Recently, we found upregulation of OPN expression in diabetic human and rat vascular walls.[10] It was also noted that high glucose concentrations stimulated OPN expression via a protein kinase C (PKC)-dependent pathway and the hexosamine pathway in cultured rat aortic SMC.[11] Furthermore, OPN was found to stimulate migration and enhance platelet-derived growth factor-mediated DNA synthesis of cultured rat aortic SMC.[10] Based on these data, we suggest that OPN plays a role in accelerated atherogenesis in diabetes mellitus.

In the present study, we further analyzed the mechanism of high glucose-induced upregulation of OPN in cultured rat aortic SMC. We show that Rho/Rho kinase pathway functions downstream of PKC and the hexosamine pathways and upstream of ERK in mediating high glucose-stimulated OPN expression.

Methods

Reagents

GGTI-298, an inhibitor of geranylgeranyltransferase I, FTI-277, an inhibitor of farnesyltransferase, Y-27632, an inhibitor of Rho-associated protein kinase, GF109203X, an inhibitor of PKC, PD98059, an inhibitor of MEK1, SB203580, an inhibitor of p38 mitogen-activated protein (MAP) kinase, and SP600125, an inhibitor of c-Jun N-terminal kinase (JNK), were purchased from Calbiochem (La Jolla, CA). Azaserine, an inhibitor of glutamine:fructose-6-phosphate amidotransferase (GFAT) was from Sigma (St. Louis, MO). The p44/42 MAP kinase assay kit, p38 MAP kinase assay kit, and SAPK/JNK assay kit were from Cell Signaling Technology (Beverly, MA). Rho activation assay kit was from UBI (Lake Placid,

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NY). pSRα-myc-RhoDA, an expression vector containing a constitutive active Rho mutant, was kindly provided by Dr Yoshimi Takai (Osaka University, Osaka, Japan). Rat OPN cDNA was from Dr Mark Thiede (Pfizer, Groton, CT). Rat glyceraldehydes-3-phosphate dehydrogenase (GAPDH) cDNA was from Dr Masashi Yamazaki (Chiba University, Chiba, Japan). Pitavastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, was from Dr Masaki Kitahara (Nissan Chemical, Saitama, Japan).

Cell Culture

Primary cultures of rat aortic SMC were isolated as described[12] by the explant method from adult male Wistar rats weighing ≈200 grams. Cells were maintained in Dulbeco modified Eagle medium containing 5.5 mmol/L glucose, 10% fetal bovine serum, and 40 μg/mL gentamicin (Schering-Plough, Kenilworth, NJ) in a humidified atmosphere at 37°C in 5% CO_2. Cells at passages 7 to 9 were used for the present experiments.

Transient Transfection

At 50% confluency in 100-mm dishes, cells were transfected with pSRα-myc-RhoDA by using Fugene 6 transfection reagent (Roche Molecular Biochemicals, Indianapolis, IN). pSRα-myc-RhoDA was mixed with Fugene 6 transfection reagent at the ratio of 1:3 and incubated at room temperature for 15 to 40 minutes. Then, cells were transfected by incubation with the mixture for 24 hours. After additional 48 hours of incubation under normal glucose concentration (5.5 mmol/L glucose), cells were processed for Northern blotting and MAP kinase activity assays.

Northern Blotting

Subconfluent cells growing in 100-mm dishes were treated with the indicated concentrations of specific inhibitors under normal or high (30 mmol/L) glucose concentrations. After 48 hours of incubation, total RNA was isolated from cells using ISOGEN (Nippon Gene, Tokyo, Japan). Northern hybridization was performed essentially as described[11] using ^{32}P-labeled rat OPN cDNA probe. The blots were stripped and subsequently re-hybridized with ^{32}P-labeled rat GAPDH cDNA probe to assess the amount of RNA loaded in each lane, or with ^{32}P-labeled Rho cDNA probe to estimate the efficiency of transfection with pSRα-myc-RhoDA. Densitometric analysis of fluorograms and autoradiograms were performed using the imaging scanner (EPSON ES 8000) with the NIH Image 1.44 software.

Assay of ERK1/2, p38 MAP Kinase and SAPK/JNK Activities

Subconfluent cells growing in 100-mm dishes were serum-starved for 24 hours and then incubated under different glucose concentrations for the indicated times. After conditioning, activities of ERK1/2 and p38 MAP kinase in cell lysates were measured by immune complex kinase assay using the p44/42 MAP kinase assay kit with an immobilized phospho p44/42 MAP kinase antibody and Elk-1 protein as substrate, or using the p38 MAP kinase assay kit with an immobilized phospho p38 MAP kinase antibody and ATF-2 protein as substrate, respectively, according to the manufacturer's instructions. After phosphorylation reactions, samples were processed for Western blotting with phospho Elk-1 antibody or phospho ATF-2 antibody. After transfection with pSRα-myc-RhoDA, JNK activity was also evaluated by immune complex kinase assay using the SAPK/JNK assay kit with an c-Jun fusion protein beads followed by Western blotting with phospho c-Jun antibody, according to manufacturer's instructions.

Rho Activation Assay

Subconfluent cells growing in 150-mm dishes were treated with the indicated concentrations of GF109203X or azaserine under high glucose concentration, or with the indicated concentrations of glucosamine under normal glucose concentration for 24 hours. Thereafter, Rho activity was measured using the Rho activation assay kit according to the manufacturer's instructions. GTP-Rho in cell lysates was adsorbed to GST-Rhotekin Rho binding domain, which binds selectively to GTP-Rho, not GDP-Rho. After precipitation, samples were processed for Western blotting with a specific anti-Rho antibody.

Western Blotting

Samples were dissolved in SDS sample buffer and boiled for 5 minutes, and the proteins were separated by SDS-PAGE on 15% (wt/vol) polyacrylamide resolving gels and electrophoretically transferred to nitrocellulose membranes (Hybond-ECL; Amersham Biosciences, Piscataway, NJ). For blocking nonspecific binding, membranes were incubated in Block Ace (Dainippon Chemicals, Tokyo, Japan) at room temperature for 1 hour. Then, the membranes were probed with the phospho Elk-1 antibody (dilution 1:1000), the phospho ATF-2 antibody (dilution 1:1000), or the anti-Rho antibody (3 μg/mL) in a dilution buffer consisting of phosphate-buffered saline containing 10% Block Ace at 4°C overnight. After being washed with phosphate-buffered saline containing 0.1% Tween-20, the membranes were incubated with an anti-rabbit IgG horseradish peroxidase-linked whole antibody (dilution 1:1000, Amersham Biosciences) in the dilution buffer at room temperature for 1 to 2 hours. After washing, the antibody binding bands were detected using an enhanced chemiluminescence system (ECL Western blotting detection reagents and analysis system; Amersham Biosciences) and visualized by exposure to Hyperfilm-ECL (Amersham Biosciences). Each experiment presented in this study was repeated at least twice under the identical conditions to confirm the reproducibility of the observations.

Results

Pitavastatin Suppresses OPN Expression Under High Glucose Concentration

Recently, we found upregulation of OPN expression in diabetic human and rat vascular walls.[10] Furthermore, oral administration of Pitavastatin, an HMG-CoA reductase inhibitor, effectively suppressed abnormally upregulated expression of OPN mRNA in the aorta and kidney of streptozotocin-induced diabetic rats.[13] These findings prompted us to examine in vitro effect of Pitavastatin on high glucose-induced upregulation of OPN expression in cultured rat aortic SMC. Cells were incubated with different concentrations of Pitavastatin at 37°C for 48 hours under high glucose concentration (30 mmol/L glucose). After incubation, the cells were processed for Northern blotting. As shown in Figure 1A, Pitavastatin dose-dependently decreased OPN mRNA level. Pitavastatin did not show cytotoxic effect at the examined doses as evaluated by trypan blue dye exclusion assay (data not shown).

Isoprenylation Is Required for OPN Expression

Inhibition of HMG-CoA reductase prevents the biosynthesis of isoprenoids, such as geranylgeranylpyrophosphate and farnesylpyrophosphate, and thereby inhibits subsequent isoprenylation. It is thus conceivable that the observed effect of Pitavastatin may result from inhibition of isoprenylation. To prove this assumption, we examined effects of inhibitors for geranylgeranyltransferase I and farnesyltransferase, GGTI-298 and FTI-277, respectively, on high glucose-induced upregulation of OPN expression in cultured rat aortic SMC. As shown in Figure 1B and C, GGTI-298 and FTI277 dose-dependently decreased OPN mRNA level under high glucose concentration, as expected.

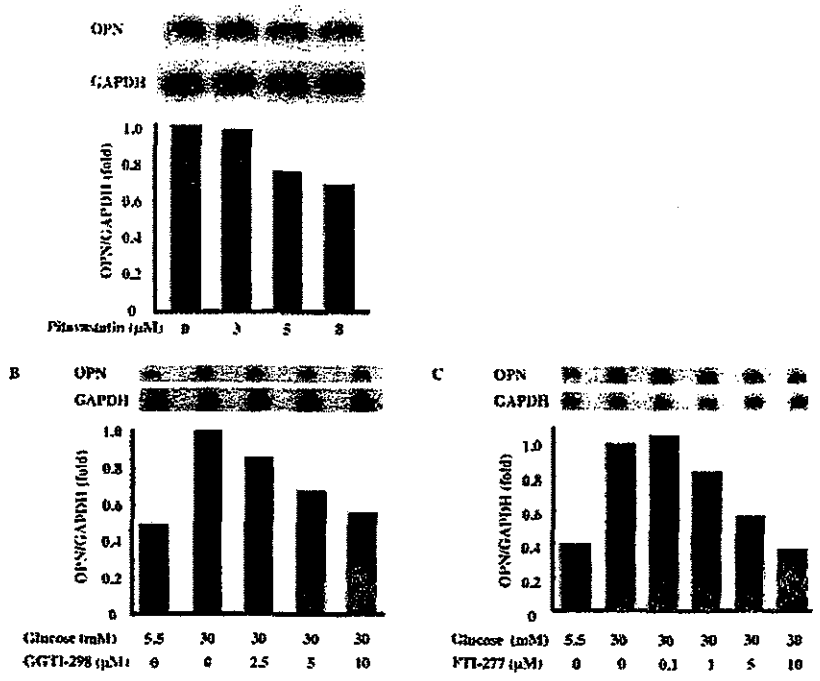

Figure 1. Effects of inhibitors for HMG-CoA reductase, geranylgeranyltransferase, and farnesyltransferase on OPN expression in cultured rat aortic SMC. After serum-starvation for 24 hours, cells were incubated with the indicated concentrations of Pitavastatin (A), GGTI-298 (B), or FTI-277 (C) in serum-free medium containing either 5.5 mmol/L or 30 mmol/L glucose for 48 hours. After incubation, cells were processed for Northern blotting with ^{32}P-labeled rat OPN and GAPDH cDNA probes. The level of OPN mRNA expression was estimated by the ratio of OPN signal to GAPDH signal. Data are expressed as fold increase relative to the value obtained in 30 mmol/L glucose without inhibitors. Data shown in this figure are representative of at least 2 independent experiments providing essentially similar results.

Rho/Rho Kinase Pathway Mediates High Glucose-Induced Upregulation of OPN Expression

It is well known that geranylgeranylation is prerequisite for Rho, a small GTP-binding protein, to exert its cellular function. Therefore, Rho seemed to be a possible candidate involved in mediating a positive signal for OPN expression. To evaluate a role of Rho, we first examined effect of an inhibitor of Rho-associated protein kinase, Y-27632, on high glucose-induced upregulation of OPN expression in cultured rat aortic SMC. As shown in Figure 2A, Y-27632 dose-dependently decreased OPN mRNA level under high glucose concentration, suggesting a critical role of Rho kinase activity in OPN expression.

Next, we examined effect of transient transfection of a constitutive active Rho mutant, pSRα-myc-RhoDA, on OPN expression in cultured rat aortic SMC. As shown in Figure 2B, transfection of pSRα-myc-RhoDA enhanced OPN mRNA expression in proportion to the efficiency of its transfection, confirming that Rho mediates a positive signal for OPN expression.

Finally, we examined effect of high glucose on Rho activation in cultured rat aortic SMC. As shown in Figure 2C, the amount of GTP-Rho in cells cultured in 30 mmol/L glucose was found to be much higher than that in 5.5 mmol/L glucose. No difference was found in total Rho protein levels between 5.5 mmol/L glucose and 30 mmol/L

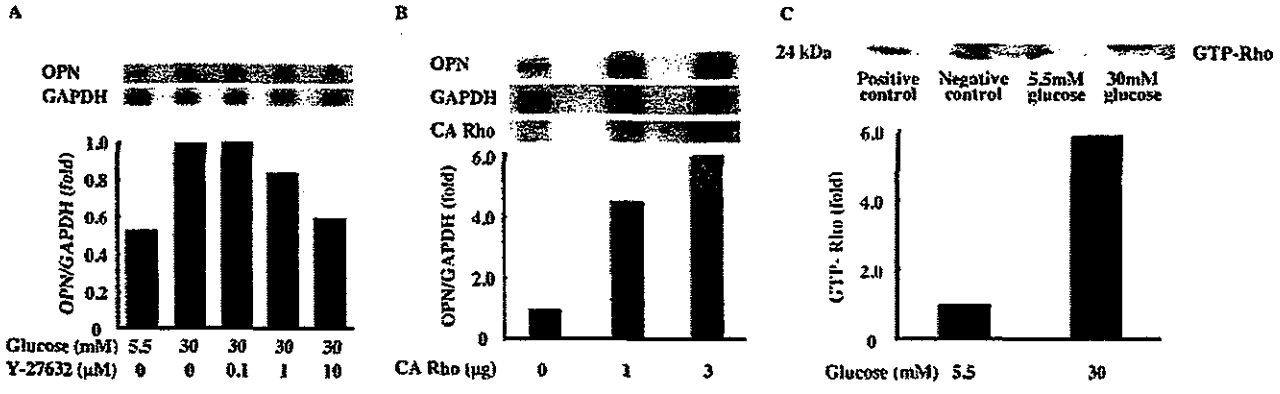

Figure 2. A, Effect of a Rho kinase inhibitor on OPN expression in cultured rat aortic SMC. After serum-starvation for 24 hours, cells were incubated with the indicated concentrations of Y-27632 in serum-free medium containing either 5.5 mmol/L or 30 mmol/L glucose for 48 hours. After incubation, cells were processed for Northern blotting as described in the legend to Figure 1. B, effect of transient transfection of a constitutive active Rho mutant (CA Rho) on OPN expression in cultured rat aortic SMC. At 50% confluency, cells were transfected with 1 to 3 μg of pSRα-myc-RhoDA and incubated for 48 hours, as described in Methods. After incubation, cells were processed for Northern blotting. The blots were re-proved with ^{32}P-labeled Rho cDNA probe to estimate the efficiency of transfection. Data are expressed as fold increase relative to the value obtained in the absence of CA Rho. C, High glucose-induced Rho activation in cultured rat aortic SMC. After serum-starvation for 24 hours, cells were incubated in serum-free medium containing either 5.5 mmol/L or 30 mmol/L glucose for 24 hours. After incubation, GTP-Rho in cell lysates was adsorbed to GST-Rhotekin Rho-binding domain and subjected to Western blotting with an anti-Rho antibody. Data are expressed as fold increase relative to the value obtained in 5.5 mmol/L glucose. Data shown in this figure are representative of at least 2 independent experiments providing essentially similar results.

glucose (data not shown). In contrast, treatment of cells with osmotic controls (5.5 mmol/L D-glucose plus 24.5 mmol/L L-glucose or 5.5 mmol/L D-glucose plus 24.5 mmol/L D-mannitol) providing an equivalent osmolarity as 30 mmol/L glucose, did not change Rho activity (data not shown), indicating that the observed enhanced effect on Rho activity is specific to glucose. Taken together, these data strongly support a notion that Rho/Rho kinase pathway mediates high glucose-induced upregulation of OPN expression.

Rho/Rho Kinase Pathway Is a Common Downstream of PKC and Hexosamine Pathways

It was previously noted that high glucose concentrations stimulated OPN expression via a PKC-dependent pathway and the hexosamine pathway in cultured rat aortic SMC.[11] Therefore, our next question was whether Rho/Rho kinase pathway functions downstream of these pathways. As shown in Figure 3A, treatment of cells with GF109203X, an inhibitor of PKC, dose-dependently inhibited high glucose-stimulated increase in Rho activity, suggesting the involvement of PKC activation in the process. Likewise, treatment with azaserine, an inhibitor of GFAT, the key enzyme of the hexosamine pathway, dose-dependently inhibited high glucose-stimulated increase in Rho activity. Total Rho protein levels were unchanged by addition of high glucose, 1 μM GF109203X or 5 μmol/L azaserine (data not shown). Furthermore, as shown in Figure 3B, glucosamine dose-dependently enhanced Rho activity. These data also suggest the involvement of the hexosamine pathway in the process.

ERK Functions Downstream of Rho in Mediating High Glucose-Induced Upregulation of OPN Expression

Small GTP-binding proteins have been demonstrated to induce a variety of responses, including activation of MAP kinase cascades in various cells. Therefore, to trace a signaling pathway that mediates OPN expression downstream of Rho, we first examined effects of inhibitors for MEK1 (PD98059), p38 MAP kinase (SB203580), and JNK (SP600125) on high glucose-induced upregulation of OPN expression in cultured rat aortic SMC. As shown in Figure 4A, PD98059 and SB203580 dose-dependently decreased OPN mRNA level under high glucose concentration, whereas SP600125 had no effect.

Next, we examined whether high glucose induces activation of ERK and p38 MAP kinase in cultured rat aortic SMC. After incubation of cells under normal (5.5 mmol/L) or high (30 mmol/L) glucose concentrations for 24 to 48 hours, activities of ERK1/2 and p38 MAP kinase were determined by immune complex kinase assay. As shown in Figure 4B, exposure to high glucose for 48 hours led to the increase in ERK activity, as assessed by phosphorylation of Elk-1, whereas activity of p38 MAP kinase, as assessed by phosphorylation of ATF-2, did not change under high glucose condition. Treatment with osmotic control (24.5 mmol/L L-glucose + 5.5 mmol/L D-glucose) had no effect on ERK activity (data not shown), indicating that the observed enhanced effect on ERK activity is specific to glucose.

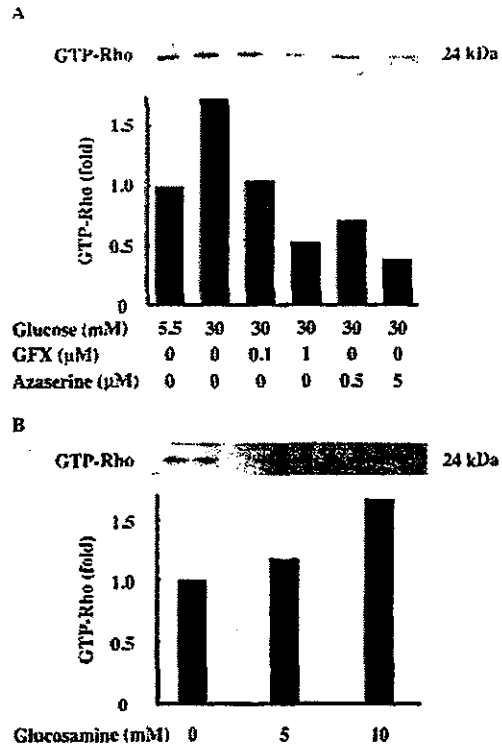

Figure 3. A, Effects of GF109203X (GFX) and azaserine on high glucose-induced Rho activation in cultured rat aortic SMC. After serum-starvation for 24 hours, cells were incubated with the indicated concentrations of GFX or azaserine in serum-free medium containing either 5.5 mmol/L or 30 mmol/L glucose for 24 hours. After incubation, cells were processed for Rho activation assay as described in the legend to Figure 2. B, Glucosamine-induced Rho activation in cultured rat aortic SMC. After serum-starvation for 24 hours, cells were incubated with the indicated concentrations of glucosamine in serum-free medium containing 5.5 mmol/L glucose for 24 hours. After incubation, cells were processed for Rho activation assay. Data are expressed as fold increase relative to the value obtained in the absence of glucosamine. Data shown in this figure are representative of at least 2 independent experiments providing essentially similar results.

Finally, to confirm that ERK functions downstream of Rho, we examined ERK activity after transient transfection of cultured rat aortic SMC with a constitutive active Rho mutant. As shown in Figure 4C, transfection of pSRα-myc-RhoDA dramatically enhanced ERK activity, whereas transfection of pSRα-myc-RhoDA did not increase either p38 MAP kinase or JNK activities. Based on these data, we concluded that ERK functions downstream of Rho in mediating high glucose-induced upregulation of OPN expression.

Discussion

In the present study, we demonstrate that Rho/Rho kinase pathway functions downstream of PKC and the hexosamine pathways and upstream of ERK in mediating high glucose-induced upregulation of OPN expression. Involvement of Rho in mediating a positive signal for OPN expression has also been reported by Chaulet et al.[14] They showed that extracellular UTP increased OPN expression in cultured rat aortic SMC and thereby induced migration of the cells. Blockade of ERK1/2 or Rho pathways led to the inhibition of

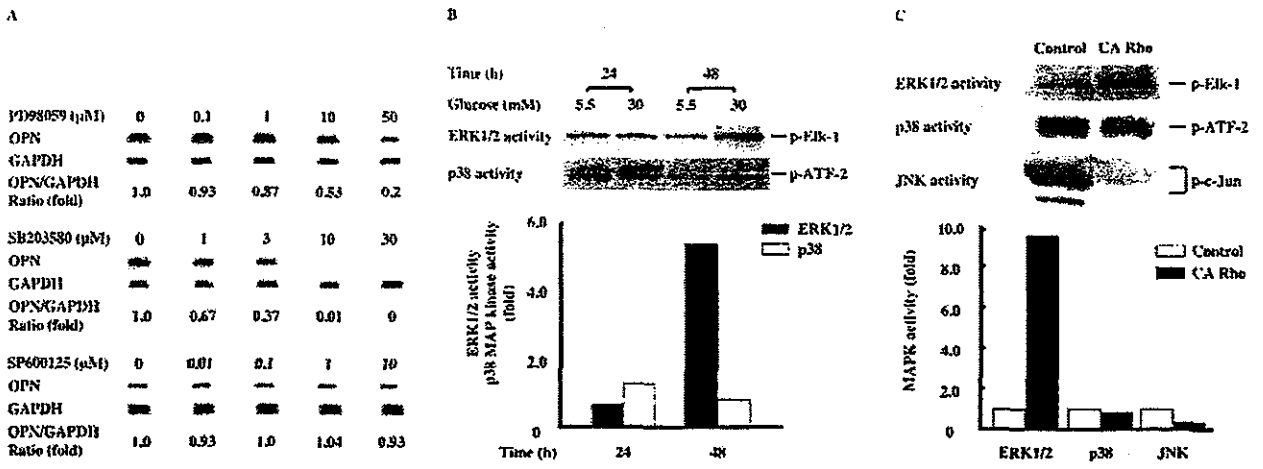

Figure 4. A, Effects of MAP kinase inhibitors on OPN expression in cultured rat aortic SMC. After serum-starvation for 24 hours, cells were incubated with the indicated concentrations of PD98059, SB203580, or SP600125 in serum-free medium containing 30 mmol/L glucose for 48 hours. After incubation, cells were processed for Northern blotting as described in the legend to Figure 1. B, High glucose-induced ERK activation in cultured rat aortic SMC. After serum-starvation for 24 hours, cells were incubated in serum-free medium containing either 5.5 mmol/L or 30 mmol/L glucose for 24 to 48 hours. After incubation, activities of ERK1/2 and p38 MAP kinase in cell lysates were measured by immune complex kinase assay with an immobilized phospho p44/42 MAP kinase antibody and Elk-1 protein as substrate, or with an immobilized phospho p38 MAP kinase antibody and ATF-2 protein as substrate, respectively. After phosphorylation reactions, samples were processed for Western blotting with phospho Elk-1 antibody or phospho ATF-2 antibody. Data are expressed as fold increase relative to the value obtained in 5.5 mmol/L glucose at the indicated times. C, Effect of transient transfection of a constitutive active Rho mutant (CA Rho) on activation of MAP kinases in cultured rat aortic SMC. Cells were transfected with 3 μg of pSRα-myc-RhoDA and incubated for 48 hours as described in the legend to Figure 2. After incubation, MAP kinase activities in cell lysates were determined. Data are expressed as fold increase relative to the value obtained in the absence of CA Rho. Double bands in the JNK activity assay correspond to 37- and 35-kilodalton forms of phosphorylated c-Jun fusion proteins. Data shown in this figure are representative of at least 2 independent experiments providing essentially similar results.

UTP-induced OPN increase and migration, demonstrating the central role of OPN in this process. The finding, together with our present observation, underscores the importance of Rho in OPN expression.

Our present finding that high glucose induces Rho activation sheds new light on the mechanism of the accelerated atherogenesis in diabetes mellitus, because involvement of Rho/Rho kinase pathway has been implicated in a wide variety of atherosclerotic processes, including neointimal formation,[15] vasospastic response,[16,17] proliferation,[18,19] migration,[19,20] and anti-apoptosis[20,21] of vascular SMC, and vascular gene expression of monocyte chemoattractant protein-1,[22] transforming growth factor-β1,[22] and inducible nitric oxide synthase.[23] Besides our present study using rat aortic SMC, high glucose-induced Rho activation was also observed in cultured rat mesangial cells[24] and in basilar artery derived from streptozotocin-induced diabetic rats.[25] It is thus conceivable that high glucose promotes diabetic vascular complications not only by upregulation of OPN but also by more diverse effects resulting from Rho activation.

It was reported that transfection of vascular SMC with the c-Ha-rasEJ oncogene induced overexpression of OPN.[26] It is well known that farnesylation is prerequisite for Ras to exert its cellular effect; therefore, our present finding that the inhibitor of farnesyltransferase, FTI-277, suppressed OPN expression might be ascribed to the inhibition of Ras function by the drug. In our previous study, however, the inhibitory effect of Pitavastatin on OPN expression in cultured rat aortic SMC was almost completely reversed by the addition of mevalonate or geranylgeranylpyrophosphate but not by farnesylpyrophosphate.[13] Studies using other types of cells, fibroblasts,[27] or keratinocytes[28] showed that transfection of dominant-negative Rho or dominant-negative Rac suppressed Ras-induced activation of Raf-MEK-ERK pathway, indicating that Ras requires either Rho or Rac function in activation of Raf-MEK-ERK pathway. Based on these findings, it is speculated that the inability of farnesylpyrophosphate to rescue the cells from the inhibition of OPN expression by Pitavastatin might be caused by suppression of Rho family function in Pitavastatin-treated cells. Further study is necessary to prove this possibility.

Acknowledgments

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References

1. Sodek J, Ganss B, McKee MD. Osteopontin. *Crit Rev Oral Biol Med.* 2000;11:279–303.
2. Xie Y, Sakatsume M, Nishi S, Narita I, Arakawa M, Gejyo F. Expression, roles, receptors, and regulation of osteopontin in the kidney. *Kidney Int.* 2001;60:1645–1657.
3. Cantor H. The role of Eta-1/osteopontin in the pathogenesis of immunological disorders. *Ann N Y Acad Sci.* 1995;760:143–150.
4. Senger DR, Perruzzi CA, Papadopoulos A. Elevated expression of secreted phosphoprotein I (osteopontin, 2ar) as a consequence of neoplastic transformation. *Anticancer Res.* 1989;9:1291–1300.
5. Craig AM, Bowden GT, Chambers AF, Spearman MA, Greenberg AH, Wright JA, McLeod M, Denhardt DT. Secreted phosphoprotein mRNA is induced during multi-stage carcinogenesis in mouse skin and correlates

with the metastatic potential of murine fibroblasts. *Int J Cancer* 1990;46: 133–137.
6. Kohri K, Nomura S, Kitamura Y, Nagata T, Yoshioka K, Iguchi M, Yamate T, Umekawa T, Suzuki Y, Sinohara H, Kurita T. Structure and expression of the mRNA encoding urinary stone protein (osteopontin). *J Biol Chem.* 1993;268:15180–15184.
7. Liaw L, Birk DE, Ballas CB, Whitsitt JS, Davidson JM, Hogan BL. Altered wound healing in mice lacking a functional osteopontin gene (spp1). *J Clin Invest.* 1998;101:1468–1478.
8. Shanahan CM, Cary NR, Metcalfe JC, Weissberg PL. High expression of genes for calcification-regulating proteins in human atherosclerotic plaques. *J Clin Invest.* 1994;93:2393–2402.
9. Liaw L, Lombardi DM, Almeida MM, Schwartz SM, deBlois D, Giachelli CM. Neutralizing antibodies directed against osteopontin inhibit rat carotid neointimal thickening after endothelial denudation. *Arterioscler Thromb Vasc Biol.* 1997;17:188–193.
10. Takemoto M, Yokote K, Nishimura M, Shigematsu T, Hasegawa T, Kon S, Uede T, Matsumoto T, Saito Y, Mori S. Enhanced expression of osteopontin in human diabetic artery and analysis of its functional role in accelerated atherogenesis. *Arterioscler Thromb Vasc Biol.* 2000;20: 624–628.
11. Takemoto M, Yokote K, Yamazaki M, Ridall AL, Butler WT, Matsumoto T, Tamura K, Saito Y, Mori S. Enhanced expression of osteopontin by high glucose in cultured rat aortic smooth muscle cells. *Biochem Biophys Res Commun.* 1999;258:722–726.
12. Morisaki N, Takahashi K, Shiina R, Zenibayashi M, Otabe M, Yoshida S, Saito Y. Platelet-derived growth factor is a potent stimulator of expression of intercellular adhesion molecule-1 in human arterial smooth muscle cells. *Biochem Biophys Res Commun.* 1994;200:612–618.
13. Takemoto M, Kitahara M, Yokote K, Asaumi S, Take A, Saito Y, Mori S. NK-104, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, reduces osteopontin expression by rat aortic smooth muscle cells. *Br J Pharmacol.* 2001;133:83–88.
14. Chaulet H, Desgranges C, Renault MA, Dupuch F, Ezan G, Peiretti F, Loirand G, Pacaud P, Gadeau AP. Extracellular nucleotides induce arterial smooth muscle cell migration via osteopontin. *Circ Res.* 2001;89: 772–778.
15. Sawada N, Itoh H, Ueyama K, Yamashita J, Doi K, Chun TH, Inoue M, Masatsugu K, Saito T, Fukunaga Y, Sakaguchi S, Arai H, Ohno N, Komeda M, Nakao K. Inhibition of Rho-associated kinase results in suppression of neointimal formation of balloon-injured arteries. *Circulation.* 2000;101:2030–2033.
16. Kandabashi T, Shimokawa H, Mukai Y, Matoba T, Kunihiro I, Morikawa K, Ito M, Takahashi S, Kaibuchi K, Takeshita A. Involvement of rho-kinase in agonists-induced contractions of arteriosclerotic human arteries. *Arterioscler Thromb Vasc Biol.* 2002;22:243–248.
17. Shimokawa H, Morishige K, Miyata K, Kandabashi T, Eto Y, Ikegaki I, Asano T, Kaibuchi K, Takeshita A. Long-term inhibition of Rho-kinase induces a regression of arteriosclerotic coronary lesions in a porcine model in vivo. *Cardiovasc Res.* 2001;51:169–177.
18. Laufs U, Marra D, Node K, Liao JK. 3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors attenuate vascular smooth muscle proliferation by preventing rho GTPase-induced down-regulation of p27(Kip1). *J Biol Chem.* 1999;274:21926–21931.
19. Seasholtz TM, Majumdar M, Kaplan DD, Brown JH. Rho and Rho kinase mediate thrombin-stimulated vascular smooth muscle cell DNA synthesis and migration. *Circ Res.* 1999;84:1186–1193.
20. Shibata R, Kai H, Seki Y, Kato S, Morimatsu M, Kaibuchi K, Imaizumi T. Role of Rho-Associated Kinase in Neointima Formation After Vascular Injury. *Circulation.* 2001;103:284–289.
21. Guijarro C, Blanco-Colio LM, Ortego M, Alonso C, Ortiz A, Plaza JJ, Diaz C, Hernandez G, Egido J. *Circ Res.* 1998;83:490–500.
22. Kataoka C, Egashira K, Inoue S, Takemoto M, Ni W, Koyanagi M, Kitamoto S, Usui M, Kaibuchi K, Shimokawa H, Takeshita A. Important role of Rho-kinase in the pathogenesis of cardiovascular inflammation and remodeling induced by long-term blockade of nitric oxide synthesis in rats. *Hypertension.* 2002;39:245–250.
23. Chen H, Ikeda U, Shimpo M, Ikeda M, Minota S, Shimada K. Fluvastatin upregulates inducible nitric oxide synthase expression in cytokine-stimulated vascular smooth muscle cells. *Hypertension.* 2000;36: 923–928.
24. Danesh F, Sadechi MM, Amro N, Phillips C, Zeng L, Lin S, Sahai A, Kanwar YS. 3-Hydroxy-3-methylglutaryl CoA reductase inhibitors prevent high glucose-induced proliferation of mesangial cells via modulation of Rho GTPase/p21 signaling pathway: implications for diabetic nephropathy. *Proc Natl Acad Sci U S A.* 2002;99:8301–8305.
25. Miao L, Calvert JW, Tang J, Zhang JH. Upregulation of small GTPase RhoA in the basilar artery from diabetic (mellitus) rats. *Life Sci.* 2002; 71:1175–1185.
26. Parrish AR, Weber TJ, Ramos KS. Osteopontin overexpression in vascular smooth muscle cells transfected with the c-Ha-rasEJ oncogene. *In Vitro Cell Dev Biol Anim.* 1997;33:584–587.
27. Li W, Chong H, Guan KL. Function of the Rho family GTPases in Ras-stimulated Raf activation. *J. Biol Chem.* 2001;276:34728–34737.
28. Mainiero F, Murgia C, Wary KK, Curatola AM, Pepe A, Blumemberg M, Westwick JK, Der CJ, Giancotti FG. The coupling of $\alpha 6\beta 4$ integrin to Ras-MAP kinase pathways mediated by Shc controls keratinocyte proliferation. *EMBO J.* 1997;16:2365–2375.