might be attributed to certain variables that may also underlie the gender difference in the association between insulin resistance, UA, and LVH. This possibility should be investigated in future studies.

We also showed that the pattern of association between BMI and low eGFR was not significantly affected by the hypertension status. Together with the finding that the association between BMI and CKD was only slightly altered when SBP was used as a covariate (Model 2 in Tables 2 and 3), this result indicates that the effect of blood pressure on the association between obesity and CKD may not be as pronounced as previously thought.

In conclusion, we analyzed cross-sectional data on 8,168 individuals (2,924 women, 5,244 men) who underwent general health screening and found that BMI showed a graded association with both low eGFR and albuminuria in men. In women, on the other hand, the second and third quartiles of WC and BMI were associated with lower prevalence of albuminuria when compared with the first BMI quartile. Obesity (BMI $\geq$30 kg/m^2) was associated with albuminuria in both genders, whereas the association between obesity and low eGFR was observed only in men. Modes of association between BMI and CKD (or its components) were similar in hypertensive and non-hypertensive individuals, especially in men. Our data showed that overweight and obesity have already become associated with an increased risk of CKD in low risk Japanese individuals, such as general health screening participants, although there is a slight gender difference.

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Are Serum Carcinoembryonic Antigen Levels Associated With Carotid Atherosclerosis in Japanese Men?

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Objective—Carcinoembryonic antigen (CEA), a serological marker of malignant tumors, may show a modest increase under some nonmalignant conditions, such as ageing and cigarette smoking. We have investigated whether serum CEA levels are associated with early carotid atherosclerosis.

Methods and Results—Cross-sectional data from 4181 male individuals who underwent general health screening were analyzed. The interquartile of cutoff values of serum CEA levels were 1.0, 1.6, and 2.5 ng/mL. Cigarette smoking was associated with increased serum CEA levels in a dose- and duration-dependent manner, and this association was more prominent in current than former smokers. Logistic regression analysis adjusted for age, body mass index, serum lipid and glucose profiles, white blood cell count, C-reactive protein, and smoking habits showed that the first, second, third, and fourth CEA quartiles were associated with carotid plaque with an odds ratio of 1 (reference), 1.25 (95% CI 1.03 to 1.52, P=0.023), 1.49 (95% CI 1.23 to 1.82 P<0.001), and 1.34 (95% CI 1.08 to 1.65; P=0.007), respectively. Although serum CEA levels were associated with metabolic syndrome, association between serum CEA and carotid plaque was significant in individuals without metabolic syndrome.

Conclusions—Serum CEA was associated with carotid atherosclerosis independently of atherogenic risk factors and markers of inflammation. Our data suggest that a slight elevation of CEA in current smokers, as well as in never smokers, may not be an innocuous observation from the viewpoint of atherosclerosis. (***Arterioscler Thromb Vasc Biol.* 2008;28:160-165.**)

Key Words: tumor marker ■ carotid atherosclerosis ■ health screening ■ cigarette smoking

Carcinoembryonic antigen (CEA) is a glycoprotein with a molecular weight of 180 to 200 kDa.[1] CEA is overexpressed in adenocarcinomas in the colon and other organs including pancreas, lung, prostate, urinary bladder, ovary, and breast; therefore, it is used as a serological marker of malignant tumors worldwide. On the other hand, serum CEA levels may increase under some nonmalignant conditions, for example, ageing, chronic renal failure, hypothyroidism, cigarette smoking, and some chronic inflammatory diseases,[2–5] although the extent of CEA elevation in such nonmalignant conditions, when present, is usually only modest. Stimulation of monocytes and macrophages with CEA may result in an increase in the production of proinflammatory cytokines,[6,7] which may subsequently upregulate adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin, on the surface of vascular endothelial cells.[7] These processes are thought to play a role in facilitating the metastasis of cancer cells. Interestingly, the early stage of atherosclerosis involves recruitment of inflammatory cells and their transendothelial migration, which is mediated by such cellular adhesion molecules on the surface of vascular endothelial cells.[8] Of note, some epidemiological studies have demonstrated a possible association between neoplastic diseases that would potently increase serum CEA and coronary artery disease.[9–11] Although modest elevation of serum CEA can be observed in apparently healthy individuals, especially in cigarette smokers,[3,12] little information is available on the possible association between serum CEA and atherosclerosis in the general population. In the current study, by analyzing the data of Japanese men who underwent general health screening, we have investigated whether there is an association between serum CEA levels and early carotid atherosclerosis.

Methods

Study Subjects

The study was approved by The Ethical Committee of Mitsui Memorial Hospital and that of University of Tokyo, Graduate School of Medicine. In Japan, regular health check-ups for employees are legally mandated. Therefore, the majority of the subjects enrolled did not have serious health problems. Between January 2003 and April 2007, 7292 subjects (2471 women, 4821 men) underwent general health screening for whom data on carotid ultrasonography and fasting insulin were available. Data on cigarette smoking habit data were collected in a self reported questionnaire, and among 4821 male

Original received July 17, 2007; final version accepted October 2, 2007.

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Arterioscler Thromb Vasc Biol. is available at http://atvb.ahajournals.org DOI: 10.1161/ATVBAHA.107.155465

Table 1. Baseline Characteristics of the Study Subjects

	Never Smoker (n=1556)	Former Smoker (n=1427)	Current Smoker (n=1198)	*P* Value
Age, years	56.5±11.5	58.8±10.2	54.4±10.2	<0.001
Body mass index, kg/m^2	23.9±3.0	24.2±2.7	24.1±2.9	0.092
Systolic blood pressure, mm Hg	128±19	131±19	125±19	<0.001
Diastolic blood pressure, mm Hg	81±12	82±11	79±12	<0.001
Laboratory data				
WBC count, $\times10^3/\mu$L	5.2±1.2	5.3±1.2	6.3±1.7	<0.001
Hemoglobin, g/dL	15.1±1.1	15.1±1.0	15.5±1.1	<0.001
Platelet count, $\times10^4/\mu$L	21.8±4.6	22.0±4.7	23.2±5.8	<0.001
LDL-cholesterol, mg/dL	128±29	129±30	127±32	0.26
HDL-cholesterol, mg/dL	55±13	57±13	53±14	<0.001
Triglycerides, mg/dL	121±72	135±97	167±139	<0.001
Uric acid, mg/dL	6.1±1.2	6.2±1.1	6.2±1.2	0.023
hsCRP, mg/dL	0.14±0.40	0.16±0.64	0.18±0.38	0.086
Fasting glucose, mg/dL	100±19	103±19	104±25	<0.001
Haemoglobin A1C, %	5.3±0.7	5.4±0.7	5.6±0.8	<0.001
Fasting insulin, μU/mL	6.8±4.7	7.0±4.4	7.2±9.0	0.36
CEA, ng/mL	1.2±0.7	1.4±1.3	2.0±1.4	<0.001
Carotid ultrasonography				
Max intima-media thickness, mm	1.27±0.65	1.36±0.72	1.32±0.71	<0.001

subjects, 4181 answered the questionnaire in full concerning the amount and the duration of smoking, and concerning the duration since they had stopped smoking at the time of the general health check if when they were former smokers. In the current study, subjects who had quit smoking for 1 month or less and those who had quit for more than 1 month before the time of the health screening were considered to be, respectively, current and former smokers, and those without a smoking history were considered to be never smokers. We were unable to identify any specific reasons for why the remaining 640 subjects failed to complete the questionnaire about their smoking status. We found that these the 640 individuals excluded were slightly but significantly older than those enrolled in the study (60±10 and 57±11 years old, respectively, $P<0.001$).

Laboratory Analysis

Blood samples were obtained from the subjects in the morning after an overnight fast, and the assays for variables analyzed in the current study were performed on the same day of blood draw without freezing the samples. Serum levels of total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) were determined enzymatically. Serum uric acid was measured by the uricase-peroxidase method, and hemoglobin A_{1C} was determined using the latex agglutination immunoassay. Plasma glucose was measured by the hexokinase method and serum insulin was measured by enzyme immunoassay. Serum CEA was measured using a commercially available immunometric chemiluminescent assay kit (Bayer Medical Co) with an interassay coefficient of variation ranging between 2.0 and 3.5%. High sensitivity C-reactive protein (hsCRP) concentration was measured by a turbidimetric immunoassay. Metabolic syndrome was diagnosed by National Cholesterol Education Program Adult Treatment Panel III[13] with a modification and it was said to be present when 3 or more of following conditions were present: (1) fasting plasma glucose (FPG) ≥110 mg/dL; (2) systolic blood pressure (SBP)/diastolic blood pressure ≥130/85 mm Hg; (3) TG ≥150 mg/dL; (4) HDL-C <40 mg/dL; and (5) BMI ≥25 kg/m^2.

Carotid Ultrasonography

Carotid artery status was assessed by high resolution B-mode ultrasonography, using a machine (Sonolayer SSA270A, Toshiba, Japan) equipped with a 7.5-MHz transducer (PLF-703ST, Toshiba). The carotid arteries were examined bilaterally at the levels of the common carotid, the bifurcation, and the internal carotid arteries from transverse and longitudinal orientations by trained sonographers. The intima-media thickness (IMT) was measured using a computer-assisted method by experienced sonographers who were unaware of the subjects' clinical and laboratory findings. Carotid intima-media wall thickening was said to occur when the IMT which was measured at the far wall of the distal 10 mm of the common carotid artery was ≥1.0 mm. Max IMT was defined as the thickest IMT in the scanned regions, and carotid plaque was defined when there was one or more focally thickened region(s) with the IMT of ≥1.1 mm.[14] The difference in the prevalence of carotid plaque in the individuals undergoing general health screening in the current study and that reported in some previous studies[15] would be explained by the difference in diagnostic criteria for carotid plaque.

Statistical Analysis

The data in this study were analyzed by the χ^2 test, ANOVA with Bonferroni post-hoc analysis, and univariate and multivariate logistic regression analysis using computer software, StatView ver. 5.0 (SAS Institute). A value of $P<0.05$ was taken to be statistically significant. Results are expressed as the mean±SD unless stated otherwise.

Results

Baseline Characteristics

The age of the subjects enrolled ranged from 21 to 89 years with a median of 57 years. Former smokers were significantly older ($P<0.001$) whereas current smokers were significantly younger ($P<0.001$) than never smokers (Table 1). Carotid plaque was found in 632/1556 (41%) of never smokers, 678/1427 (48%) of former smokers, and 539/1198 (45%) of current smokers. Carotid intima-media thickening was found in 125/1556 (8%) of never smokers, 161/1427 (11%) of former smokers, and 114/1198 (10%) of current smokers. Pearson's correlation coefficients between CEA and various

Table 2. Pearson's Correlation Coefficients Between Serum CEA Levels and Various Parameters

Variable	Coefficient	*P* Value
Age, years	0.16	<0.001
Body mass index, kg/m^2	−0.06	<0.001
Systolic blood pressure, mm Hg	0.04	0.044
Diastolic blood pressure, mm Hg	0.01	0.89
Laboratory data		
WBC count, ×10^3/mL	0.16	<0.001
Hemoglobin, g/dL	0.02	0.64
Platelet count, ×10^4/mL	−0.03	0.11
LDL-cholesterol, mg/dL	−0.01	0.64
HDL-cholesterol, mg/dL	0.02	0.50
Triglycerides, mg/dL	0.00	0.99
Uric acid, mg/dL	0.01	0.66
hsCRP, mg/dL	0.03	0.22
Fasting glucose, mg/dL	0.14	<0.001
Haemoglobin A1C, %	0.19	<0.001
Fasting insulin, μU/mL	0.03	0.26
Carotid ultrasonography		
Max intima-media thickness, mm	0.10	<0.001

variables are described in Table 2. Age, WBC count, FPG, hemoglobin A_{1c} showed only a weak correlation with CEA with the correlation coefficients ranging between 0.1 and 0.2; however, the correlation between serum CEA and hsCRP was not statistically significant (Table 2). The median (range) of the first to fourth quartiles of CEA values was 0.5 (0.5 to 0.7), 1.0 (0.8 to 1.2), 1.6 (1.3 to 1.9), and 2.5 (2.0 to 37.2) ng/mL, respectively. Of the 4181 subjects enrolled, 489 (12%) and 36 (0.9%) had CEA levels greater than 2.5 ng/mL (upper normal limit) and 5.0 ng/mL, respectively.

Smoking Habits and Serum CEA Levels

In current smokers, CEA levels increased according to the amount and duration of smoking, whereas this tendency was less apparent in former smokers (Figure). In smokers, the prevalence of the highest CEA quartile was found to increase with the daily number of cigarettes smoked as well as with smoking duration (Table 3). This trend was more prominent in current smokers than in former smokers. The odds ratio for the highest CEA quartile tended to get smaller with the length of smoking cessation in former smokers; however, after adjusting for age, former smokers who had last smoked ≥5 years ago at the time of assessment were found to still have a greater prevalence of the highest serum CEA quartile as compared with never smokers (Table 3), in agreement with a previous observation.[16]

Association Between CEA and Carotid Atherosclerosis

Of the individuals in the first to fourth CEA quartiles, carotid plaque was found in 343/1051 (33%), 613/1071 (43%), 532/1074 (50%), and 516/985 (52%), respectively, and carotid intima-media thickening was found in 73/1051 (7%), 92/1071 (9%), 101/1074 (9%), and 134/985 (14%), respectively. When the lowest serum CEA quartile was used as a reference, logistic regression analysis showed that the higher serum CEA quartiles were positively associated with carotid plaque even after adjusting for age, SBP, lipid and glucose data, smoking status, and the inflammatory markers, WBC and hsCRP (Model 4 in Table 4). In this model, hsCRP was also significantly associated with carotid plaque with an odds ratio of 1.26 (95% CI 1.04 to 1.54, per 1 mg/dL increase, P=0.019). In contrast, after adjusting for the same variables, the association between the second, third, and fourth serum CEA quartiles with carotid intima-media thickening was not significant with an odds ratio of 0.92 (95% CI 0.66 to 1.29), 0.84 (95% CI 0.60 to 1.18), and 1.10 (0.79 to 1.55), respectively. Of 4173 study subjects, 3890 (93%) had a

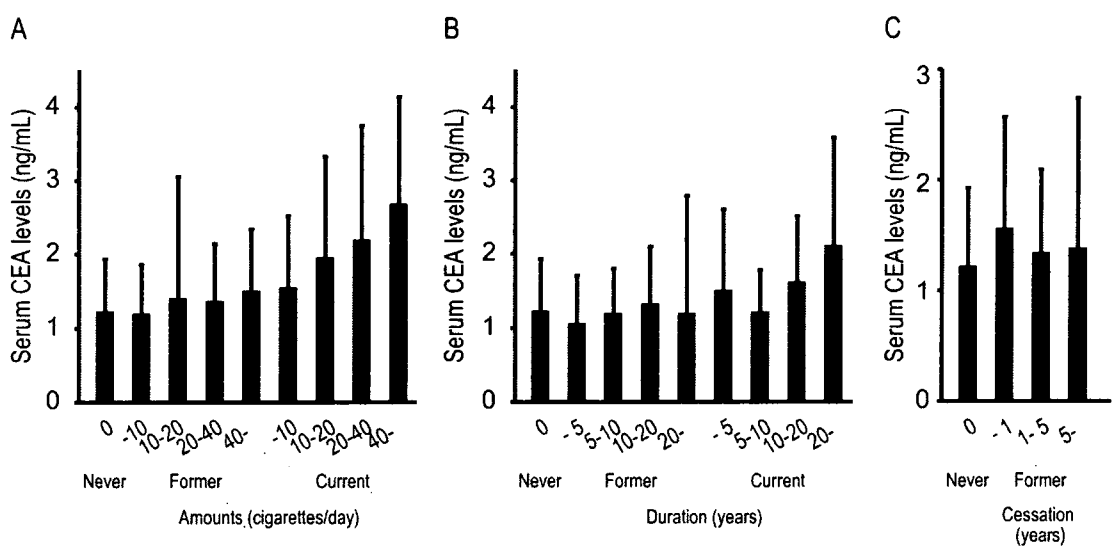

Figure. Serum CEA levels according to smoking habits. A, According to daily number of cigarettes smoked. B, According to duration of cigarette smoking. C, According to cessation period.

Table 3. Unadjusted and Age-adjusted Association of Smoking Habits With the Highest Serum CEA Quartile

Smoking Status	Odds Ratio (95% CI) Unadjusted	*P*	Odds Ratio (95% CI) Adjusted for Age	*P*
Amount of smoking				
Never smoking	1.00	...	1.00	...
Former smoking§				
<10 (cigarettes/d)	0.83 (0.52–1.33)	0.45	0.85 (0.53–1.37)	0.51
10–19	1.53 (1.18–1.98)	0.001	1.43 (1.10–1.86)	0.008
20–39	1.43 (1.09–1.87)	0.010	1.31 (1.00–1.72)	0.054
40≤	2.02 (1.46–2.81)	<0.001	1.76 (1.26–2.47)	<0.001
Current smoking§				
<10	1.84 (1.28–2.65)	0.001	2.02 (1.40–2.93)	<0.001
10–19	3.94 (3.11–4.98)	<0.001	4.52 (3.54–5.76)	<0.001
20–39	5.35 (4.23–6.76)	<0.001	6.40 (5.02–8.17)	<0.001
40≤	9.93 (6.33–15.59)	<0.001	11.32 (7.14–17.94)	<0.001
Duration of smoking				
Never smoking	1.00	...	1.00	...
Former smoking§				
<5y	0.75 (0.40–1.43)	0.38	0.87 (0.45–1.66)	0.67
5–9	0.59 (0.34–1.00)	0.048	0.66 (0.39–1.13)	0.13
10–19	1.40 (1.06–1.85)	0.019	1.39 (1.05–1.85)	0.023
20≤	1.92 (1.53–2.40)	<0.001	1.59 (1.27–2.01)	<0.001
Current smoking§				
<5y	1.64 (0.54–4.98)	0.39	1.83 (0.59–5.67)	0.30
5–9	0.66 (0.23–1.88)	0.44	0.93 (0.32–2.67)	0.89
10–19	2.20 (1.48–3.27)	<0.001	3.28 (2.16–5.00)	<0.001
20≤	4.98 (4.11–6.02)	<0.001	5.37 (4.42–6.53)	<0.001
Years of cessation				
Never smoking	1.00	...	1.00	...
Former smoking§				
Last smoked <1y ago	2.75 (1.75–4.33)	<0.001	3.07 (1.92–4.90)	<0.001
Last smoked 1–4y ago	1.30 (0.91–1.84)	0.15	1.42 (0.99–2.02)	0.055
Last smoked ≥5y ago	1.42 (1.15–1.75)	0.001	1.24 (1.00–1.54)	0.046

§Never smoking was used as a reference.

fasting glucose level of less than 140 mg/dL and were not taking antidiabetic medication. In these subjects, the second, third, and fourth serum CEA quartiles were associated with carotid plaque with an odds ratio of 1.28 (95% CI 1.05 to 1.57, $P=0.013$), 1.49 (95% CI 1.22 to 1.82, $P=0.0001$), and 1.32 (1.06 to 1.64, $P=0.013$), respectively, after adjusting for HOMA-IR and the covariates used in Model 4 of Table 4. The odds ratio of each serum CEA quartile for the carotid plaque in never, former, and current smokers is described in Table 5. The association between the highest serum CEA quartile and carotid plaque did not reach statistical significance after this subdivision.

Metabolic syndrome was found in 783 (19%) individuals. After adjusting for age, logistic regression analysis showed that the first to fourth CEA quartiles were associated with metabolic syndrome with an odds ratio of 1 (reference), 1.04 (95% CI 0.83 to 1.31, $P=0.72$), 1.15 (95%CI 0.92 to 1.44, $P=0.21$), and 1.40 (95% CI 1.12 to 1.75, $P=0.004$), respectively. Among the 3383 individuals who did not have metabolic syndrome, the first to fourth serum CEA quartiles were associated with carotid plaque with an odds ratio of 1 (reference), 1.04 (95% CI 0.83 to 1.31, $P=0.72$), 1.48 (95% CI 1.20 to 1.84, $P<0.001$), and 1.30 (95% CI 1.02 to 1.64, $P=0.031$), respectively. We then investigated the association between serum CEA and increased insulin resistance, defined here as the highest HOMA-IR quartile (HOMA-IR >2.15), in 3890 individuals who had a fasting glucose level of less than 140 mg/dL and were not taking antidiabetic medication. We found that the first to fourth CEA quartiles were associated with the highest HOMA-IR quartile with an odds ratio of 1 (reference), 1.26 (95% CI 1.02 to 1.56, $P=0.032$), 1.30 (95% CI 1.05 to 1.64, $P=0.015$), and 1.32 (95% CI 1.05 to 1.65, $P=0.015$), respectively.

Discussion

In the current study, we have investigated the possible association between serum CEA levels and carotid atherosclerosis by analyzing the data of 4181 male individuals who

Table 4. Logistic Regression Analysis of the CEA Quartiles as Independent Variables and Carotid Plaque as a Dependent Variable

CEA quartiles	Odds Ratio for Carotid Plaque	*P* Value
Model 1		
Q1	1.00	...
Q2	1.54 (1.29–1.84)	<0.001
Q3	2.03 (1.70–2.42)	<0.001
Q4	2.27 (1.90–2.72)	<0.001
Model 2		
Q1	1.00	...
Q2	1.29 (1.07–1.56)	0.009
Q3	1.62 (1.34–1.95)	<0.001
Q4	1.59 (1.31–1.93)	<0.001
Model 3		
Q1	1.00	...
Q2	1.26 (1.04–1.53)	0.017
Q3	1.50 (1.24–1.82)	<0.001
Q4	1.38 (1.13–1.70)	0.002
Model 4		
Q1	1.00	...
Q2	1.25 (1.03–1.52)	0.023
Q3	1.49 (1.23–1.82)	<0.001
Q4	1.34 (1.08–1.65)	0.007

Model 1: Unadjusted.
Model 2: Adjusted for age.
Model 3: Adjusted for age, SBP, and smoking status.
Model 4: Adjusted for age, SBP, FPG, BMI, LDL-C, HDL-C, TG, smoking status, WBC, and hsCRP.

underwent general health screening. As compared with the lowest serum CEA quartile, individuals in the 3 higher serum CEA quartiles had significantly increased prevalence of carotid plaque (Table 3). This association remained statistically significant even after adjusting for age, SBP, FPG, BMI, LDL-C, HDL-C, TG, smoking status, WBC, and hsCRP. In the smokers, especially the current smokers, serum CEA levels were increased according to the daily number of cigarettes smoked and duration of smoking (Figure, Table 3). We found previously that circulating WBC count, a marker for systemic inflammation, was also increased according to the amount and duration of smoking,[17,18] and increased WBC count was a risk factor for carotid atherosclerosis independent of other conventional risk factors.[19] Therefore, the association between serum CEA levels and carotid atherosclerosis might be confounded by that between circulating WBC count and atherosclerosis. However, the association between CEA and carotid plaque remained statistically significant after adjustment for WBC count and hsCRP (Model 4, Table 4).

What is the possible underlying mechanism, if present, which would explain the observed link between serum CEA and carotid plaque? First, serum CEA was associated with metabolic syndrome and increased insulin resistance. Because both of these conditions can increase the risk for carotid atherosclerosis, increased insulin resistance or metabolic syndrome may explain the observed link between serum CEA and carotid plaque. On the other hand, the association

Table 5. Logistic Regression Analysis of the CEA Quartiles as Independent Variables and Carotid Plaque or Carotid Intima-Media Thickening as a Dependent Variable According to Smoking Status

CEA Quartiles	Odds Ratio for Carotid Plaque	*P* Value
Never smoker		
Q1	1.00	...
Q2	1.17 (0.87–1.56)	0.30
Q3	1.51 (1.11–2.04)	0.008
Q4	1.42 (0.98–2.04)	0.063
Former smoker		
Q1	1.00	...
Q2	1.36 (1.00–1.86)	0.049
Q3	1.41 (1.02–1.94)	0.039
Q4	1.15 (0.81–1.64)	0.43
Current smoker		
Q1	1.00	...
Q2	1.26 (0.77–2.08)	0.36
Q3	1.74 (1.10–2.75)	0.018
Q4	1.46 (0.94–2.28)	0.096

Adjusted for age, SBP, FPG, BMI, LDL-C, HDL-C, TG, WBC, and hsCRP.

between serum CEA and carotid plaque remained statistically significant after adjustment for HOMA-IR, and this association was found to be statistically significant among individuals who did not have metabolic syndrome, suggesting that the association was, at least in part, independent of increased insulin resistance or metabolic syndrome. Second, several previous studies have suggested that CEA may stimulate the monocytes/macrophages to release proinflammatory cytokines, which eventually lead to the induction of adhesion molecules on the surface of vascular endothelial cells, which may facilitate the metastasis of malignant cells.[6,7,20] Interestingly, it has been found that ICAM-1 and VCAM-1 levels correlate with serum CEA levels in colorectal cancer patients.[21] Recruitment of inflammatory cells from the circulation after the induction of adhesion molecules on the surface of vascular endothelial cells is postulated to be an early phase of atherosclerosis[8]; therefore, enhanced expression of certain adhesion molecules on vascular cells may explain the observed link between CEA and carotid plaque. Third, serum CEA may be increased in patients with chronic inflammatory disorders[22] such as inflammatory bowel disease,[23,24] collagen disease, and chronic viral hepatitis,[25] although this idea remains controversial.[26] Patients with such chronic inflammatory diseases may have an increased risk of carotid atherosclerosis.[27–29] It is possible that a similar immune-inflammatory reaction, such as activation of the CD40/CD40 ligand system, might play a crucial role in both atherosclerosis and inflammatory diseases.[30,31] Whether serum CEA levels are associated with blood levels or membrane-bound levels of adhesion molecules and CD40 ligand should be investigated in future studies. Although cigarette smoking increases serum CEA levels without evidence of malignant diseases,[2] elevated serum CEA levels may be associated with a more accelerated decline in the percent forced expiratory volume in one second (FEV1%) value among smokers.[32]

Thus, our data may provide additional evidence that an increase in serum CEA levels among cigarette smokers may not be an innocuous observation also from the viewpoint of atherosclerosis.

This study has several potential limitations. First, we analyzed only men in the current study, because the number of female subjects who had a smoking history was much smaller during the study period. Second, because of the cross-sectional nature of the study, we cannot determine whether there is a causal or resultant relationship between the elevation of serum CEA and carotid plaque. Third, in addition to cigarette smoking, serum CEA is known to be elevated in other nonmalignant conditions, such as hypothyroidism,[4] and end-stage lung diseases.[33] We did not include these disorders as confounding variables, because their prevalence is considered to be very low among the study population.

In conclusion, we have shown that cigarette smoking increases serum levels of CEA in a dose- and duration-dependent manner in Japanese men who underwent general health screening. The increase in serum CEA was found to be associated with an increased prevalence of carotid plaque independent of blood pressure, fasting glucose, serum lipids, and inflammatory markers. Our data suggested that an elevation of CEA in smokers may not be an innocuous observation from the viewpoint of atherosclerosis.

Acknowledgments

The work was supported in part by a Grant from the Smoking Research Foundation.

Disclosures

None.

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Critical Role of Bone Marrow Angiotensin II Type 1 Receptor in the Pathogenesis of Atherosclerosis in Apolipoprotein E–Deficient Mice

Daiju Fukuda, Masataka Sata, Nobukazu Ishizaka, Ryozo Nagai

Objective—It is suggested that the angiotensin II (Ang II)–Ang II type 1 receptor (AT1R) pathway plays a pivotal role in the pathogenesis of atherosclerosis. Recently, bone marrow (BM) cells were reported to express AT1R. Here, we investigated the role of AT1R in BM in the pathogenesis of atherosclerosis.

Methods and Results—Genetic ablation or pharmacological blockade of AT1R led to a significant reduction and stabilization of atherosclerotic lesions in $ApoE^{-/-}$ mice. To elucidate the role of AT1R in BM, we generated several BM chimeric mice. Ang II promoted atherosclerosis progression in the BM chimeric mice that had AT1aR in BM, regardless of the absence of AT1aR in the recipient vasculature ($P<0.05$). BM chimeric mice whose BM AT1aR was disrupted showed significantly less atherosclerotic lesions in aorta ($P<0.05$) and more stable plaque with reduced accumulation of BM-derived cells compared with BM chimeric mice that had AT1aR-positive BM. Most of the BM-derived cells in atheroma were positive for a macrophage marker and expressed matrix metalloproteinase (MMP)-9 and monocyte chemoattractant protein-1.

Conclusions—Our findings suggest that AT1R in BM plays an important role in the pathogenesis of atherosclerosis. (***Arterioscler Thromb Vasc Biol.* 2008;28:90-96.**)

Key Words: angiotensin II type I receptor ■ bone marrow ■ atherosclerosis ■ MMP-9 ■ MCP-1

It is a widely accepted view that atherosclerosis is a chronic inflammatory disease.[1] Although multifactorial in etiology, vascular inflammation produces atherosclerosis through the continuous recruitment of circulating leukocytes into the vessel wall and by contributing to an oxidant-rich inflammatory milieu. Recent advances in immunology have dissected several molecular pathways that induce and promote inflammatory responses in atherosclerotic lesions.[1]

The renin–angiotensin system (RAS) has been suggested to play a role in the pathogenesis of atherosclerosis by simulating a series of coordinated cellular and molecular events observed in atherosclerotic lesions.[2] Angiotensin II (Ang II) induces the production of reactive oxygen species and stimulates the expression of adhesion molecules and chemokines, leading to endothelial dysfunction, adhesion and invasion of leukocytes, lipid deposition, and smooth muscle cell proliferation.[2] These observations suggest that local effects of an activated RAS in the vessel wall play a central role in the pathogenesis of chronic vascular inflammation by directly acting on resident vascular cells.

The RAS is reported to be involved in the maintenance of cell proliferation and organ remodeling under physiological or pathophysiological conditions in many tissues other than the cardiovascular system.[2] It was suggested that an activated RAS has local effects in bone marrow (BM), which contributes to the regulation of both normal and malignant hematologic processes.[3] It was demonstrated that Ang II increases hematopoietic progenitor cell proliferation.[4] Recently, Cassis et al reported that Ang II promoted vascular pathology via Ang II type 1a receptor (AT1aR) and that AT1aR expressed on infiltrating cells exerted modest regulation of Ang II-induced atherosclerosis in LDL receptor–deficient mice.[20] It was suggested that the presence of AT1aR in resident tissue was required for the initiation of Ang II–induced atherosclerosis and aneurysm formation.

Although ApoE-deficient and LDL receptor–deficient mice are the most widely used mouse models for atherosclerosis, they differ markedly in lesion type and in their susceptibility to different atherogenic stimuli.[5] Here, we tested the hypothesis that local effects of an activated RAS, especially AT1aR, in BM may play a role in the pathogenesis of atherosclerosis in ApoE-deficient mice. Analyses of BM chimeric mice revealed that AT1aR in BM plays an important role in progression and destabilization of atherosclerotic plaques. We performed a detailed analysis of cellular components of plaque composition and investigated the molecular

Original received April 23, 2007; final version accepted October 12, 2007.

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Arterioscler Thromb Vasc Biol. **is available at http://atvb.ahajournals.org** **DOI: 10.1161/ATVBAHA.107.152363**

mechanism by which BM AT1aR contributes to progression and destabilization of atherosclerotic plaque.

Methods

Animals

ApoE-deficient ($ApoE^{-/-}$) mice were originally purchased from Jackson Laboratory. Mice deficient in AT1aR, the type 1a receptor of Ang II, ($AT1aR^{-/-}$) were described previously.[6] GFP mice were described previously.[7] Double knockout mice deficient in ApoE and AT1aR were generated by cross-breeding $ApoE^{-/-}$ mice and $AT1aR^{-/-}$ mice. Furthermore, we also generated GFP-positive $ApoE^{-/-}AT1aR^{-/-}$ mice ($ApoE^{-/-}AT1aR^{+/+}GFP^{+/+}$ mice) and GFP-positive $ApoE^{-/-}AT1aR^{-/-}$ mice ($ApoE^{-/-}AT1aR^{-/-}GFP^{+/+}$ mice). We administered 10 mg/kg/d olmesartan, an AT1R blocker, or 30 mg/kg/d hydralazine to 6-week-old male $ApoE^{-/-}$ mice by gavage every day for 24 weeks. An osmotic mini-pump (Alzet) was used to infuse Ang II (5 mg/kg/d).

Bone Marrow Transplantation

Bone marrow transplantation (BMT) was performed as described previously.[7] At 4 weeks after BMT, all animals were started on a Western type diet. We used only BM chimeric mice, in which more than 80% of BM had been replaced by donor BM. All experimental procedures and protocols were approved by the Animal Care and Use Committee of the University of Tokyo.

Preparation of Aortas and Atherosclerotic Lesions

Lipid deposition was quantified by en face analysis of the aorta as previously described.[8] The atherosclerotic lesions in aortic root were analyzed by Oil red O staining, Sirius red staining, and immunohistochemistry as previously described.[9]

Laser Microdissection and Quantitative Real-Time Polymerase Chain Reaction

The atherosclerotic lesions were collected from the aortic root with a Laser Microdissection System (AS LMD, Leica) according to the manufacturer's instructions. Total RNA was isolated with the use of the RNeasy MicroKit (QIAGEN). First strand cDNA was synthesized from the obtained total RNA using a Quantitect Reverse Transcription Kit (QIAGEN) for quantitative real-time polymerase chain reaction (PCR).

Statistical Analysis

Numerical values are expressed as mean±SEM. Comparison of parameters between 2 groups was performed by unpaired Student *t* test. A value of $P<0.05$ was considered significant.

For further details, please refer to the supplemental materials (available online at http://atvb.ahajournals.org).

Results

Effects of Genetic Ablation or Pharmacological Blockade of AT1R on Atherosclerotic Plaque Formation

We generated $ApoE^{-/-}AT1aR^{-/-}$ double knockout mice by cross-breeding $ApoE^{-/-}AT1aR^{+/+}$ mice and $ApoE^{+/+}AT1aR^{-/-}$ mice. We compared atherosclerotic lesion progression between male $ApoE^{-/-}AT1aR^{+/+}$ mice (n=9) and $ApoE^{-/-}AT1aR^{-/-}$ mice (n=7) fed normal chow. As previously reported, systolic blood pressure was significantly lower in $ApoE^{-/-}AT1aR^{-/-}$ mice (90.1±2.9 mm Hg) than in $ApoE^{-/-}AT1aR^{+/+}$ mice (101.1±3.4 mm Hg, $P=0.03$). Plasma total cholesterol level was significantly higher in $ApoE^{-/-}AT1aR^{-/-}$ mice (911±102 mg/dL) than in $ApoE^{-/-}AT1aR^{+/+}$ mice (513±41 mg/dL, $P=0.006$). At 32 weeks of age, en face Sudan IV staining of the aortic arch revealed a

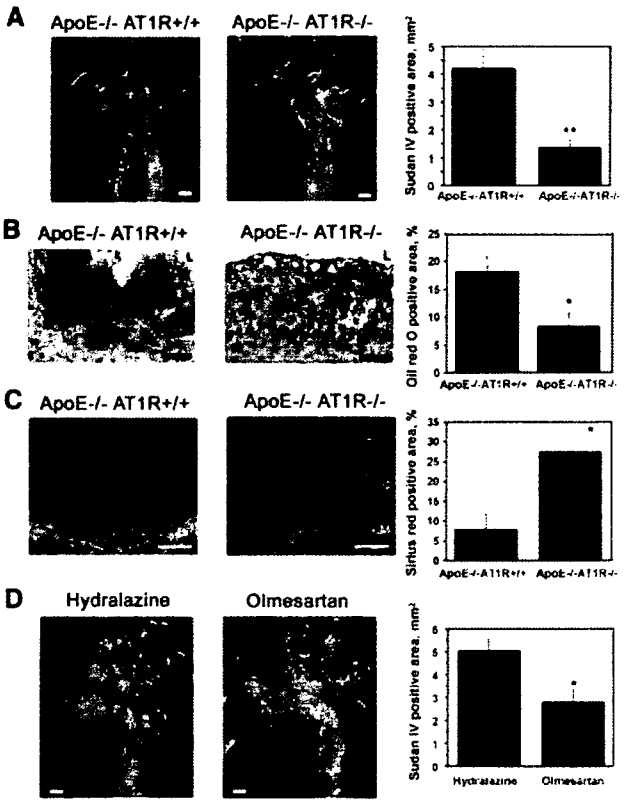

Figure 1. Effects of genetic ablation or pharmacological blockade of AT1R on atherosclerosis. A, En face Sudan IV staining of aortic arch in $ApoE^{-/-}AT1aR^{+/-}$ and $ApoE^{-/-}AT1aR^{-/-}$ mice. Bar, 1 mm. B, Oil red O staining. Bar, 50 μm. C, Sirius red staining. Bar, 50 μm. D, Quantification of lesions in mice treated with olmesartan or hydralazine. Bar, 1 mm. *$P<0.05$, **$P<0.01$. L indicates lumen; M, media.

significant reduction in atherosclerotic lesion formation in $ApoE^{-/-}AT1aR^{-/-}$ mice (1.4±0.3 versus 4.2±0.7 mm^2, $P=0.003$; Figure 1A). Furthermore, Oil red O staining and Sirius red staining of atherosclerotic lesions in the aortic root revealed significantly decreased lipid deposition (8.4±2.4 versus 18.1±2.8%, $P=0.04$) and increased collagen content (27.4±5.3 versus 7.9±4.0%, $P=0.03$) in the plaques of $ApoE^{-/-}AT1aR^{-/-}$ mice compared with those of $ApoE^{-/-}AT1aR^{+/+}$ mice (Figure 1B and 1C). We infused Ang II (5 mg/kg/d) or vehicle into the 20-week-old mice for 2 weeks. In $ApoE^{-/-}AT1aR^{+/+}$ mice, Ang II markedly accelerated atherosclerotic lesion formation in aortic arch (9.3±1.4 versus 2.1±0.4 mm^2, $P=0.0001$) associated with significant elevation in systolic blood pressure (143.0±3.2 versus 95.5±2.6 mm Hg, $P<0.0001$). On the other hand, in $ApoE^{-/-}AT1aR^{-/-}$ mice, there was no significant difference in atherosclerotic lesion area (0.8±0.3 versus 1.5±0.5 mm^2, $P=0.20$) or in blood pressure (92.1±4.2 versus 80.5±4.5 mm Hg, $P=0.09$) between the Ang II–treated and vehicle-treated groups.

Next, we administered 10 mg/kg/d olmesartan (n=6), an AT1R blocker, or 30 mg/kg/d hydralazine (n=6) to 6-week-old male $ApoE^{-/-}AT1aR^{+/+}$ mice fed a Western-type diet every day by gavage for 24 weeks. There was no significant difference between the 2 groups in systolic blood pressure

(hydralazine, 64.7±2.6 versus olmesartan, 61.7±1.4 mm Hg; P=0.52) or plasma total cholesterol level (hydralazine, 527±33 versus olmesartan, 523±27 mg/dL, P=0.93). Consistent with the effects of genetic ablation of AT1aR, en face Sudan IV staining of the aortic arch revealed significant suppression of atherosclerotic lesion progression by olmesartan (2.8±0.6 versus 5.1±0.5 mm^2, P=0.01) (Figure 1D). Furthermore, Oil red O staining of the plaques in the aortic root revealed that olmesartan decreased lipid content (7.3±1.3 versus 14.5±2.9%, P=0.048) with increased collagen content (38.7±4.3 versus 23.1±4.5%, P=0.03) as detected by Sirius red staining.

Effects of Restoration of BM AT1aR on Atherosclerosis in ApoE$^{-/-}$AT1aR$^{-/-}$ Mice

To evaluate the potential contribution of AT1aR in BM to the pathogenesis of atherosclerosis, we generated several combinations of BM chimeric mice. We performed BMT from ApoE$^{-/-}$AT1aR$^{-/-}$ mice to ApoE$^{-/-}$AT1aR$^{-/-}$ mice at 10 to 14 weeks of age. We also performed BMT from ApoE$^{-/-}$AT1aR$^{-/+}$ mice to ApoE$^{-/-}$AT1aR$^{-/-}$ mice at the same age. These BM chimeric mice had AT1aR in BM, but not in their innate vascular cells. At 12 weeks after BMT, white blood cell count was similar between the AT1aR$^{-/-}$ recipients repopulated with AT1aR$^{-/-}$ BM and AT1aR$^{+/+}$ BM (5.6±0.4 versus 4.9±0.5×10^3/μL, P=0.28). From 12 weeks after BMT, we infused 5 mg/kg/d Ang II or vehicle into these BM chimeric mice for 8 weeks using an osmotic mini-pump. Ang II infusion into these BM chimeric mice elevated blood pressure significantly compared with vehicle infusion, though these mice had no AT1aR in their vasculature. There was no significant difference in blood pressure or in plasma cholesterol level between Ang II-treated AT1aR$^{-/-}$ recipient mice repopulated with AT1aR$^{-/-}$ BM and Ang II-treated AT1aR$^{-/-}$ recipient mice repopulated with AT1aR$^{+/+}$ BM (systolic blood pressure, 97.0±6.2 versus 107.4±3.6 mm Hg, P=0.16; total cholesterol level, 728±50 versus 650±54 mg/dL, P=0.32). After 8 weeks of infusion, en face Sudan IV staining of the aortic arch revealed that atherosclerotic lesions in AT1aR$^{-/-}$ recipients with AT1aR$^{+/+}$ BM (n=8) were significantly larger than those in AT1aR$^{-/-}$ recipients with AT1aR$^{-/-}$ BM (n=7; 1.9±0.5 versus 0.6±0.2 mm^2, P=0.03; Figure 2). Histological analysis of atherosclerotic lesions in the aortic root revealed that lipid deposition detected by Oil red O staining was accelerated in AT1aR$^{-/-}$ recipients with AT1aR$^{+/+}$ BM compared with those in AT1aR$^{-/-}$ recipients with AT1aR$^{-/-}$ BM (12.1±2.2 versus 5.5±1.4%, P=0.03). Collagen content demonstrated by Sirius red staining was decreased in AT1aR$^{-/-}$ recipients with AT1aR$^{+/+}$ BM compared with that in AT1aR$^{-/-}$ recipients with AT1aR$^{-/-}$ BM (9.7±1.5 versus 18.2±2.6%, P=0.01). We measured mRNA expression of MMP-9, MCP-1, and vascular cell adhesion molecule (VCAM)-1 in the plaques by means of a laser microdissection system and quantitative RT-PCR. MMP-9 expression in AT1aR$^{-/-}$ recipients with AT1aR$^{+/+}$ BM tended to be greater compared with that in AT1aR$^{-/-}$ recipients with AT1aR$^{-/-}$ BM (3.5±1.5 versus 0.7±0.2 [arbitrary unit], P=0.11). There was no statistical difference in MCP-1 (7.6±2.4 versus 3.6±0.8 [arbitrary unit], P=0.15) or

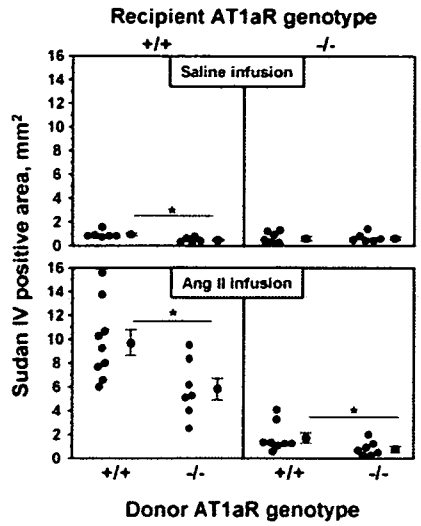

Figure 2. Atherosclerotic lesion development after BMT. BMT was performed between ApoE$^{-/-}$AT1aR$^{+/+}$ and ApoE$^{-/-}$AT1aR$^{-/-}$ mice. Ang II or vehicle was infused for 8 weeks starting at 12 weeks after BMT. Lesions were quantified after en face Sudan IV staining of the aortic arch. Circles represent size in individual mice. The right circles with bars represent means. *P<0.05.

VCAM-1 (5.6±0.4 versus 6.0±0.3 [arbitrary unit], P=0.58) expression. Among the vehicle treated mice (n=7 for each group), collagen content in atheroma in AT1aR$^{-/-}$ recipients with AT1aR$^{+/+}$ BM was significantly decreased compared with that in AT1aR$^{-/-}$ recipients with AT1aR$^{-/-}$ BM, although atherosclerotic lesion area in aorta (Figure 2), lipid content in atheroma and RNA expression in the lesion were similar (supplemental Table I). Taken together, these results suggest that BM transplantation from AT1aR$^{+/-}$ donors to AT1aR$^{-/-}$ recipients could restore Ang II–induced acceleration of atherosclerosis and plaque destabilization.

Effects of Targeted Disruption of BM AT1aR on Atherosclerosis in ApoE$^{-/-}$AT1aR$^{+/+}$ Mice

Next, to keep track of BM-derived cells in the process of atherosclerotic lesion progression, we replaced BM of ApoE$^{-/-}$AT1aR$^{+/+}$ mice with that of ApoE$^{-/-}$AT1aR$^{-/-}$GFP$^{-/+}$ mice or ApoE$^{-/-}$AT1aR$^{+/+}$GFP$^{-/+}$ mice at 10 weeks of age. The former BM chimeric mice lacked AT1aR only in BM, and the latter chimeric mice had AT1aR in both BM and the vasculature. At 12 weeks after BMT, white blood cell count was similar between the AT1aR$^{+/+}$ recipient mice repopulated with AT1aR$^{+/+}$ BM and the AT1aR$^{+/+}$ recipient mice repopulated with AT1aR$^{-/-}$ BM (6.9±0.6 versus 6.3±0.8×10^3/μL, P=0.52). In these BM chimeric mice, we compared the effects of Ang II on atherosclerotic lesion formation. We infused Ang II from 12 weeks after BMT. After 8 weeks infusion, en face Sudan IV staining of the aortic arch revealed that acceleration of atherosclerotic lesion was significantly attenuated in the AT1aR$^{+/+}$ recipients repopulated with AT1aR$^{-/-}$ BM (n=7) compared with that in the AT1aR$^{-/-}$ recipients repopulated with AT1aR$^{-/-}$ BM (n=9) (5.8±0.9 versus 9.8±1.1 mm^2, P=0.02; Figures 2 and 3A), with reduced accumulation of GFP-positive cells (5.8±0.3 versus 9.3±1.3 mm^2, P=0.03; Figure 3A). In these

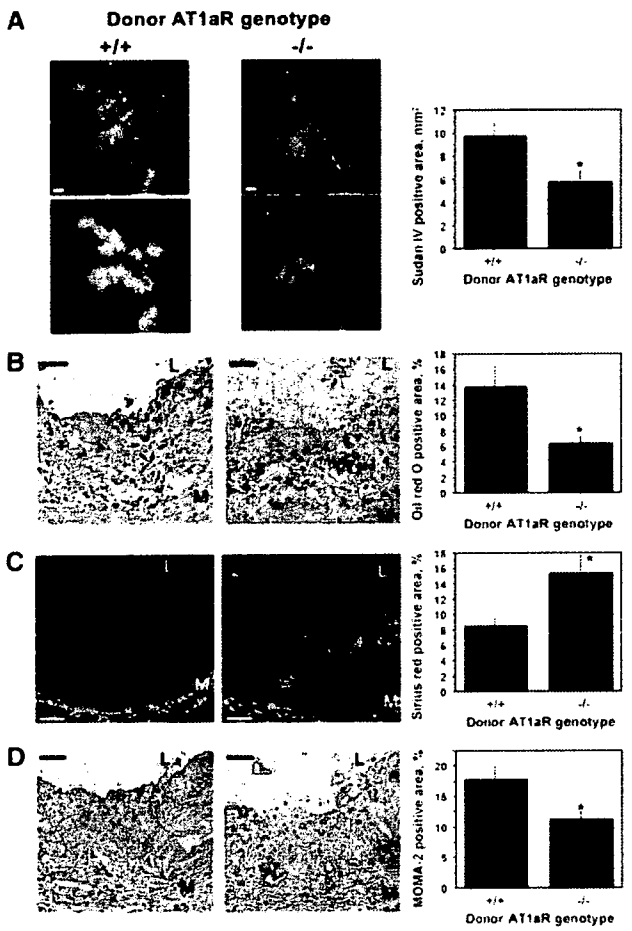

Figure 3. Targeted disruption of AT1aR in BM-ameliorated atherosclerotic lesion progression and destabilization in ApoE$^{-/-}$ AT1aR$^{+/+}$ mice. A, En face Sudan IV staining of aorta. GFP signal indicates accumulation of BM-derived cells. Bar, 1 mm. B, Oil red O staining of the lesions in aortic root. Bar, 50 μm. C, Sirius red staining. Bar, 50 μm. D, Anti-MOMA-2 immunohistochemistry. Bar, 50 μm. L indicates lumen; M, media. *P<0.05.

2 types of BM chimeric mice, there was no significant difference in blood pressure (136.9±4.7 versus 141.7±3.1 mm Hg, P=0.40) or in total cholesterol level (1019±87 versus 912±76 mg/dL, P=0.37). In atherosclerotic plaques in the aortic root in the AT1aR$^{+/-}$ recipients with AT1aR$^{-/-}$ BM showed significantly reduced lipid deposition (6.5±0.8 versus 13.8±2.7%, P=0.04) and increased collagen content (15.3±2.3 versus 8.6±1.9%, P=0.01) compared with that in AT1aR$^{+/-}$ recipients with AT1aR$^{+/+}$ BM (Figure 3B and 3C). These results suggest that BM-derived cells may play a role in the pathogenesis of accelerated atherosclerosis induced by Ang II. Infiltration of macrophages into the lesions was significantly reduced in the AT1aR$^{+/+}$ recipients with AT1aR$^{-/-}$ BM compared with that in the AT1aR$^{+/-}$ recipient with AT1aR$^{+/+}$ BM as demonstrated by immunostaining for MOMA-2 (11.2±1.2 versus 17.8±2.1%, P=0.02; Figure 3D). MMP-9 RNA expression in the AT1aR$^{+/+}$ recipient with AT1aR$^{-/-}$ BM in atheroma was significantly reduced compared with that in the AT1aR$^{+/+}$ recipient with AT1aR$^{+/+}$ BM (0.9±0.7 versus 8.9±3.1 [arbitrary unit], P=0.03). In these BM chimeric mice, there was no significant difference in MCP-1 (4.6±0.4 versus 4.7±0.6 [arbitrary unit], P=0.87) nor VCAM-1 (5.4±0.3 versus 5.3±0.4 [arbitrary unit], P=0.87) expression.

Next, we infused vehicle into these BM chimeric mice for 8 weeks. There was no significant difference in blood pressure between the AT1aR$^{+/+}$ recipients with AT1aR$^{+/+}$ BM and those with AT1aR$^{-/-}$ BM. Similar to Ang II–treated mice, atherosclerotic lesion of aorta was significantly attenuated in the AT1aR$^{+/+}$ recipients with AT1aR$^{-/-}$ BM (n=5) compared with that in the AT1aR$^{+/+}$ recipients with AT1aR$^{+/+}$ BM (n=6; Figure 2). However, lipid deposition, collagen content, and RNA expression in atheroma were similar in the AT1aR$^{+/+}$ recipients repopulated with AT1aR$^{+/+}$ BM or AT1aR$^{-/-}$ BM (supplemental Table I).

To investigate how AT1aR positive BM cells contribute to the pathogenesis of atherosclerosis, we examined gene expression in the plaques by means of a laser microdissection system and quantitative RT-PCR (n=4 in each group) at 4 weeks after Ang II infusion (Figure 4A). Expressions of MMP-9 (3.0-fold, P=0.04) and MCP-1 (7.1-fold, P=0.02) in the AT1aR$^{+/+}$ recipients repopulated with AT1aR$^{+/+}$ BM was significantly greater than those in the AT1aR$^{+/+}$ recipients repopulated with AT1aR$^{-/-}$ BM. On the other hand, there was no significant difference in VCAM-1 expression between the 2 BM chimeric mice. Accumulation of BM-derived GFP-positive cells was significantly accelerated in AT1aR$^{+/+}$ recipients with AT1aR$^{-/+}$ BM (n=8) compared with that in AT1aR$^{+/+}$ recipients with AT1aR$^{-/-}$ BM (n=7; 60.3±3.8 versus 38.4±1.9%, P=0.0003). Most of the BM-derived cells were positive for a macrophage marker (Figure 4B). Furthermore, the percentage of BM-derived GFP-positive cells among the MMP-9–positive cells (72.7±6.3 versus 38.2±1.9%, P=0.0003) or the MCP-1–positive cells (55.1±2.8 versus 42.5±4.8%, P=0.10) was greater in the AT1aR$^{+/+}$ recipient with AT1aR$^{+/+}$ BM than in the AT1aR$^{+/+}$ recipient with AT1aR$^{-/-}$ BM (Figure 4C and 4D).

Discussion

In this study, we demonstrated that genetic ablation or pharmacological blockade of AT1R effectively suppressed atherosclerotic lesion formation with more stabilized morphological characteristics of the plaque. We found that AT1aR-positive BM cells accelerated atherosclerotic lesion progression and plaque destabilization, even if the recipient vasculature cells did not express AT1aR. On the other hand, lack of AT1aR in BM cells decreased atherosclerotic lesion progression and stabilized plaques with or without Ang II infusion despite the existence of AT1aR in vascular cells. Histological studies revealed that accumulation of BM-derived cells in atherosclerotic lesions was enhanced when AT1aR was expressed in BM cells. Moreover, the existence of AT1aR in BM significantly increased the expression of MMP-9 and MCP-1 in atherosclerotic plaques. The percentage of BM-derived cells among the MCP-1– or MMP-9–expressing cells in the lesions was decreased by the disruption of AT1aR in BM. Most of the BM-derived cells accumulated in the lesions were positive for a macrophage marker. Taken together, our present study demonstrated

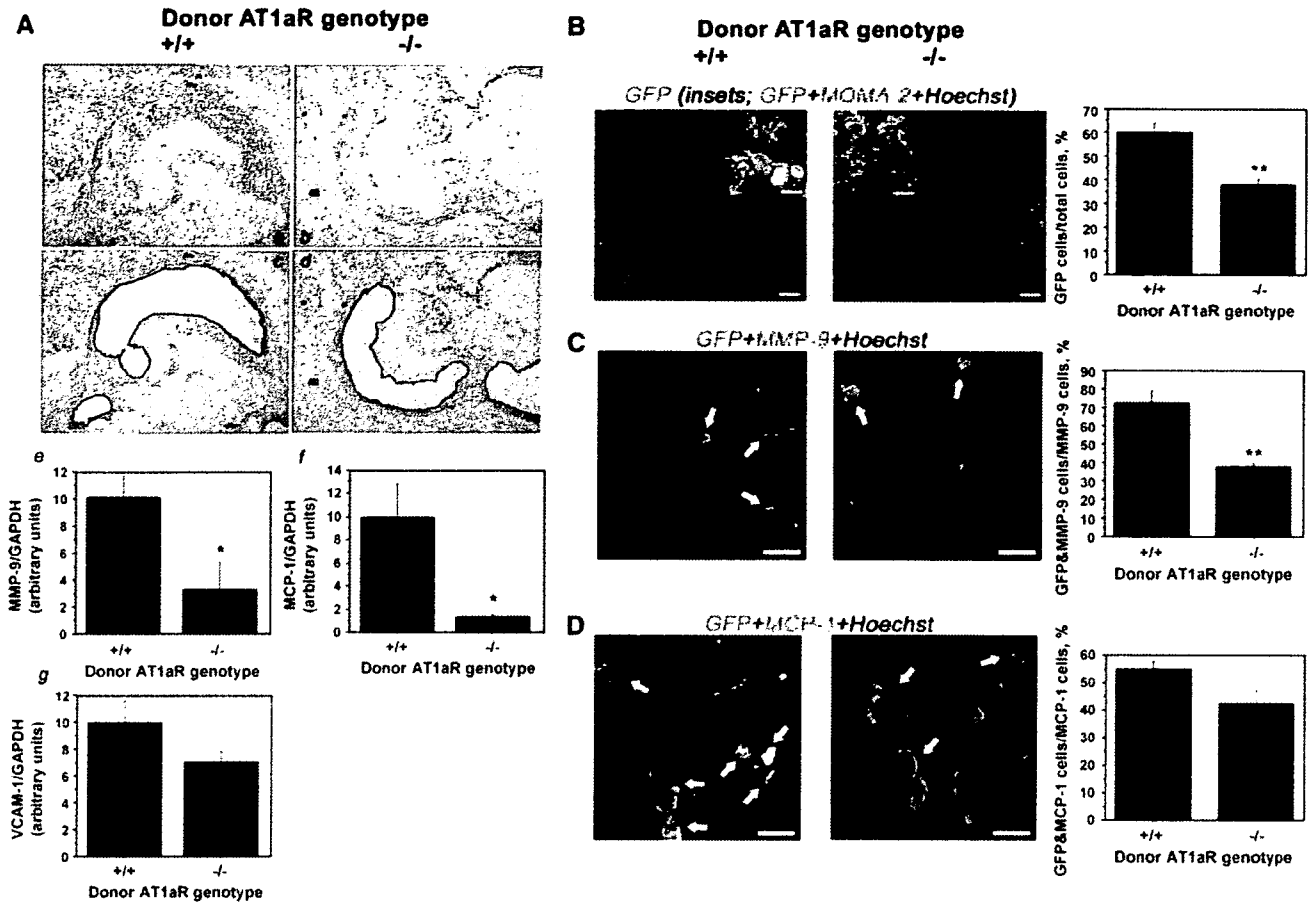

Figure 4. Targeted disruption of AT1aR in BM cells altered characteristics of atherosclerotic lesions. Characteristics of the plaque at 4 weeks after Ang II infusion in AT1aR$^{+/-}$ recipients. A, Total RNA was isolated from the lesions collected with the use of a laser microdissection system. B through D, Double immunofluorescent studies. B, Bar, 100 μm. Bar, 10 μm (insets). C and D, Bar, 10 μm. *P<0.05, **P<0.001.

functional contribution of the AT1aR in BM to the pathogenesis of atherosclerosis in vivo.

The RAS has been considered to be a circulating hormonal system that regulates blood pressure and flow. Recent studies have provided evidence for local effects of an activated RAS, particularly in the cardiac, vascular, and renal systems.[10] It is now well established that Ang II has significant proinflammatory actions on the vessel wall, leading to progression of atherosclerosis.[11] It is well known that there are 2 different types of Ang II receptors, AT1R and AT2R, in mammals. Both AT1R and AT2R have been identified in the vessel wall. In rodents, 2 AT1R subtypes, AT1aR and AT1bR, have been identified. In the vasculature, AT1aR is predominant and mediates most of the physiological and pathophysiological responses to Ang II in mice.[6] There is increasing evidence of cross-talk between RAS and dyslipidemia in atherogenesis.[12] It was demonstrated that hypercholesterolemia stimulates angiotensin peptide synthesis[12] and increased the density of AT1R,[13] suggesting that Ang II–AT1R pathway may meditate, at least in part, the atherogenic effects of hypercholesterolemia. Consistently, previous reports demonstrated that inhibition of AT1R-signaling reduces atherosclerosis.[14] The greatest AT1R density has been found on vascular smooth muscle cells and endothelial cells. Thus, the antiatherogenic effects of AT1R blockade are thought to result from inhibition of AT1R-mediated signaling in resident vascular cells.

Recent reports suggest that local effects of an activated RAS exist in BM and functions to promote differentiation and proliferation of BM cells.[4] Our preliminary study revealed that AT1aR was abundantly expressed in BM, whereas other receptors were hardly detected (supplemental Figure IA). We also found that AT1aR could be detected in atherosclerotic lesions in the AT1aR$^{-/-}$ recipients repopulated with AT1aR$^{+/+}$ BM (supplemental Figure IB). Thus, we here focused on AT1aR in BM, although it is plausible that other receptors of Ang II may participate in the atherogenic effects of Ang II.

Previous reports have demonstrated that AT1R in the vasculature mediates upregulation of adhesion molecules and chemokines, thus promoting infiltration of inflammatory cells into the vessel wall.[15] MCP-1 in vascular cells is one of the essential inflammatory mediators in Ang II–induced progression of atherosclerosis.[16] Several reports have demonstrated that monocytes/macrophages release MCP-1 through activation of the Ang II–AT1R pathway in vitro.[17] Our results showed the expression of MCP-1 from BM-derived cells in plaques. Selective disruption of AT1aR in BM significantly decreased MCP-1 expression in plaques in ApoE$^{-/-}$AT1aR$^{+/-}$

mice that received Ang II infusion for 4 weeks. It was suggested that AT1aR-positive BM-derived cells themselves could be a source of MCP-1 in plaques. As well as MCP-1, MMPs are demonstrated to be expressed in atherosclerotic lesions.[18] Especially, MMP-9 is important for the resorption of extracellular matrix and contributes to progression and destabilization of atherosclerosis. An AT1R antagonist is reported to inhibit MMP-9 expression in a mouse model of atherosclerosis.[19] Our present results showed that AT1aR-positive BM-derived cells are an important source of MMP-9.

Recently, when this study was being conducted, Cassis et al reported that Ang II (1.0 μg/kg/min) promotes vascular pathology via AT1aR in LDL receptor–deficient mice.[20] Consistent with our findings, repopulation of AT1aR$^{+/+}$ mice with AT1aR$^{-/-}$ BM resulted in modest reductions in Ang II–induced atherosclerosis.[20] Here, we confirm the importance of AT1aR in BM. In addition, we demonstrate that AT1aR-positive BM cells not only accelerate accumulation of BM-derived cells in the lesions through MCP-1 expression, but also contribute to plaque progression and destabilization by secretion of MMP-9, at least in part. Thus, our study significantly extends the findings of Cassis et al and provides novel insights into the mechanism by which Ang II promotes atherosclerosis progression and instability.

Unexpectedly, Cassis et al found that AT1aR$^{-/-}$ recipient mice were dramatically protected from Ang II (1.44 mg/kg/d for 4 weeks)-induced vascular pathologies irrespective of BM donor genotype, suggesting that the presence of AT1aR in resident tissue is required for the initiation of Ang II–induced atherosclerosis.[20] In this study, we also found that atherosclerosis development was notably retarded in AT1aR$^{-/-}$ recipients with vehicle infusion regardless of the existence of AT1aR in BM. However, Ang II infusion at a higher dose for a longer period (5.0 mg/kg/d for 8 weeks) could promote atherosclerosis significantly even in AT1aR$^{-/-}$ recipients when the BM cells were repopulated with AT1aR$^{+/+}$ BM. Others reported that there are several differences in the pathogenesis of dyslipidemia and atherosclerosis between ApoE$^{-/-}$ and LDL-R$^{-/-}$ mice.[5] Moreover, we infused Ang II into BM chimeric mice at 12 weeks after BMT, whereas Cassis et al started Ang II treatment at 7 weeks after BMT. In our study, blood cell count at 12 weeks after BMT was similar among 4 BMT groups. At 40 weeks after BMT, the blood cell count and the BM cell composition were also similar in the AT1aR$^{+/-}$ recipients repopulated with AT1aR$^{+/+}$ BM and AT1aR$^{-/-}$ BM (supplemental Table II). However, many reports documented that only the limited number of engrafted cells are matured with skewed population frequencies at 4 to 6 weeks after BMT, although high numbers of donor cells can be observed in peripheral blood at 4 to 6 weeks after BMT.[21] Assays of immune function indicate deficient functions at earlier time points after BMT. Thus, the differences in the type of hypercholesterolemic mice, the protocol of Ang II infusion, and the timing of Ang II infusion relative to the BMT might lead to the different results by us and by Cassis et al.

The dose of Ang II used in our study might be high compared with those used in previous studies. However, 5 mg/kg/d Ang II did not cause apparent toxic effects, such as increased mortality (supplemental Table III), changes in plasma lipid profile, or body weight loss, in the BM chimeric mice. Moreover, in our preliminary study, 3.0 mg/kg/d of Ang II infusion was not sufficient for blood pressure elevation and acceleration of atherosclerosis even in AT1aR$^{+/+}$ recipients repopulated with AT1aR$^{+/+}$ BM (Fukuda and Sata, unpublished data), suggesting that higher dose of Ang II would be required to evaluate the effects of Ang II in BM chimeric ApoE$^{-/-}$ mice repopulated with exogenous BM after lethal irradiation. Therefore, we chose the dose of 5 mg/kg/d of Ang II in this study.

Unexpectedly, in our study, ApoE$^{-/-}$AT1aR$^{-/-}$ mice showed significantly higher total cholesterol level than that in ApoE$^{-/-}$AT1aR$^{+/+}$ mice. This was inconsistent with previous reports.[22] Analysis of lipid profile showed similar pattern in ApoE$^{-/-}$AT1aR$^{+/+}$ mice and ApoE$^{-/-}$AT1aR$^{-/-}$ mice (data not shown). ApoE$^{-/-}$AT1aR$^{-/-}$ mice showed significantly lower blood pressure compared with ApoE$^{-/-}$AT1aR$^{+/+}$ mice, consistent with a previous report.[22] It could be possible that alteration of blood pressure and lipid level may affect the development of atherosclerosis in ApoE$^{-/-}$AT1aR$^{-/-}$ mice. However, in our experiment on pharmacological blockade of AT1R, there were no significant difference in blood pressure and cholesterol level between olmesartan-treated mice and hydralazine-treated mice. In our studies with BM chimeric mice, there was no significant difference in blood pressure or plasma cholesterol level in AT1aR$^{-/-}$ recipients or AT1aR$^{+/+}$ recipients regardless of BM AT1aR genotype. Therefore, our data obtained with the BM chimeric mice appear to be independent of blood pressure and plasma cholesterol level.

In summary, our results suggest that AT1aR expressed not only on vascular cells but also on BM-derived cells plays a role in the pathogenesis of atherosclerosis, at least in part. Therefore, blockade of AT1R not only in vascular cells but also in BM could be an important strategy to prevent the progression and destabilization of atherosclerotic plaques.

Sources of Funding

This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology and the Ministry of Health, Labor, and Welfare of Japan. Dr Fukuda is supported by a Research Fellowship from the Japan Society for the Promotion of Science for Young Scientists.

Disclosures

None.

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Information Up-to-Date 758

至適，正常，正常高値血圧者のメタボリックシンドロームと頸動脈硬化

Metabolic syndrome may not associate with carotid plaque in subjects with optimal, normal, or high-normal blood pressure.
Ishizaka N *et al*：*Hypertension* **48**：411-417, 2006

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はじめに

メタボリックシンドロームを有する症例は，同シンドロームを有していない症例に比較して，心血管病や脳卒中のリスクが高い．それゆえこの概念は，ハイリスク症例の特定のみならず，疾病予防に対する意識改善のためにも有用な指標であると考えられている．一方，高血圧や糖尿病に対し，すでに投薬治療を受けている症例は，メタボリックシンドロームの有無にかかわらず心血管病に対するリスクが高いことは周知のとおりである．よって，血圧，糖・脂質代謝の異常が存在しないか，あっても軽度の症例において，メタボリックシンドロームの有無により心血管病リスクが比較的高いと考えられる症例を特定できるのかどうか，という点が問題となるが，そのような観点からの検討はほとんどなされていなかった．

本論文では，人間ドック受診症例のうち，血圧が収縮期140 mmHg未満かつ90 mmHg未満の症例を対象に，メタボリックシンドロームを有している場合に早期動脈硬化病変の頻度が上昇しているかどうかを横断的研究で調査している．

対　象

三井記念病院総合健診センターを受診した症例のうち，頸動脈超音波によるスクリーニングを含む健康評価を受けた症例から，以下の方法に示す条件にもとづいて対象群を絞り込んだ．なお，糖尿病に対して投薬を受けている症例は対象から除外している．

方　法

メタボリックシンドロームの診断基準は，NCEP ATP IIIによる基準のうち，腹囲基準をBMI≥25 kg/m²で置き換えたものを使用した．頸動脈プラークの診断は1.3 mm以上の局所的な内膜中膜コンプレックスの肥厚と定義した．また，壁肥厚は，内膜中膜コンプレックス1.0 mm以上の肥厚と定義した．

本研究では，頸動脈超音波によるスクリーニングを含む健康評価を受けた8,143症例から，以下の条件絞り込みの程度により，3つの対象群を設定している．

①血圧が140/90 mmHg未満であること

②降圧薬を投与されていないこと

③食前血糖が126 mg/dl未満であること

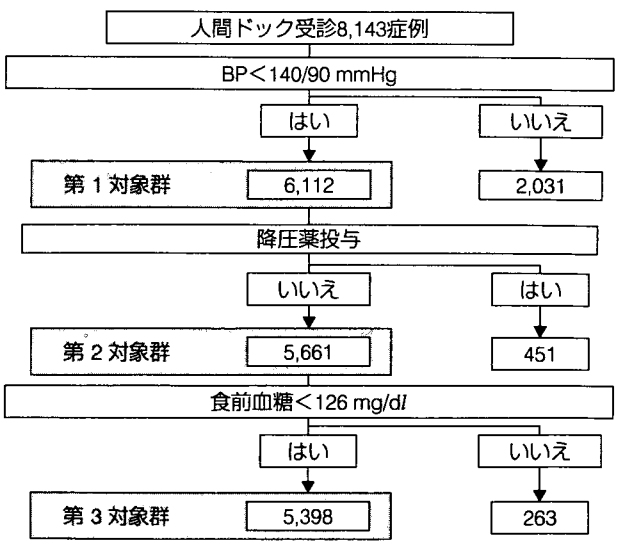

図 1．対象症例

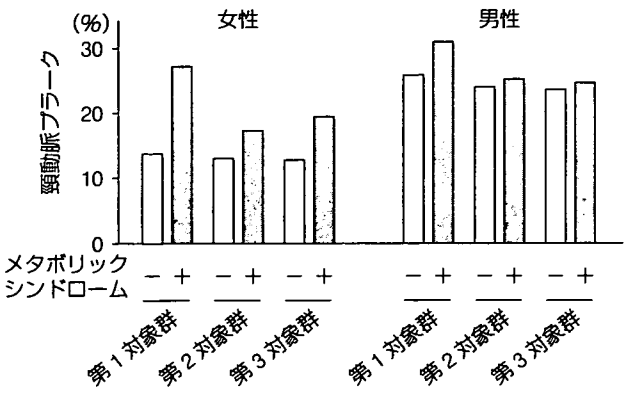

図 2. 各群における頸動脈プラークの頻度

上記の①を満たすものを第1対象群(6,112症例)，①，②を満たすものを第2対象群(5,661症例)，①～③を満たすものを第3対象群（5,398症例）とした（**図1**）.

結　果

いずれの対象群においても性別にかかわらず，メタボリックシンドロームを有する症例において，頸動脈プラーク，および，頸動脈肥厚の頻度が高かったが，降圧薬を投与されていない症例のみからなる第2，第3対象群では，第1対象群よりもメタボリックシンドロームの有無による頸動脈プラークの頻度の差は，かなり小さくなっていた（**図2**）.

つぎに，メタボリックシンドロームと頸動脈プラークの関連に関して，ロジスティック回帰分析で検討した．年齢補正の検討では，男女とも，第1対象群においては，メタボリックシンドロームが頸動脈プラークの独立した危険因子となっていたが，第2，第3対象群では，この関連は統計的には有意なものではなかった（**表1**）．いずれの対象群においても，メタボリックシンドロームは頸動脈壁肥厚との関連は有意なレベルに達しなかった.

表 1. 頸動脈プラークに対するメタボリックシンドロームのオッズ比

	女性		男性	
	オッズ比 (95% CI)	p 値	オッズ比 (95% CI)	p 値
第1対象群				
補正なし	2.37 (1.43-3.94)	<0.001	1.29 (1.06-1.58)	<0.05
年齢で補正	2.11 (1.22-3.64)	<0.01	1.39 (1.12-1.73)	<0.01
年齢，総コレステロール値，喫煙状況で補正	2.04 (1.18-3.52)	<0.05	1.33 (1.07-1.66)	<0.05
第2対象群				
補正なし	1.43 (0.74-2.78)	0.29	1.07 (0.84-1.35)	0.59
年齢で補正	1.29 (0.63-1.63)	0.48	1.21 (0.94-1.56)	0.15
年齢，総コレステロール値，喫煙状況で補正	1.23 (0.60-2.50)	0.58	1.14 (0.88-1.48)	0.31
第3対象群				
補正なし	1.67 (0.85-3.26)	0.14	1.06 (0.81-1.38)	0.69
年齢で補正	1.54 (0.75-3.19)	0.24	1.25 (0.94-1.68)	0.13
年齢，総コレステロール値，喫煙状況で補正	1.47 (0.71-3.04)	0.30	1.18 (0.88-1.58)	0.28

まとめ

人間ドック受診症例のうち，降圧薬の投与を受けてなく，血圧が140/90 mmHg未満の症例では，メタボリックシンドロームという指標により早期動脈硬化病変のリスクが比較的高い症例を同定することはむずかしい可能性が示唆された．そのような対象は，人間ドック受診症例の約7割（＝5,661/8,143）であった.

比較的，血圧，糖・代謝異常の程度が軽い症例においては，メタボリックシンドロームの有無にかかわらず，個々の危険因子に対して対処することが望ましいと考えられる.

Higher serum uric acid is associated with increased arterial stiffness in Japanese individuals.

Ishizaka N, Ishizaka Y, Toda E, Hashimoto H, Nagai R, Yamakado M.
Atherosclerosis 2007; 192: 131-7. PMID: 16716328.

日本人において血清尿酸値の上昇は動脈スティフネスの亢進と関連している

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石坂裕子／遠田栄一／橋本英樹／永井良三／山門　実

背景・目的

尿酸値が高い症例では心血管疾患が増加していることが知られている。高尿酸血症症例では、また、高血圧、糖・脂質代謝障害、あるいは、メタボリックシンドロームを合併する頻度が高いことが知られており、これらの危険因子が、高尿酸血症と動脈硬化を介在している可能性もある。一方、これらのトラディショナルな心血管危険因子とは独立に、高尿酸血症が、冠動脈や頸動脈における動脈硬化の頻度増加に関連している可能性があることも報告されている。最近、本邦からも、メタボリックシンドロームが脈波伝播速度（PWV）の増大と関連しているという報告がなされている。今回われわれは、尿酸値の高い症例においてPWVが増大している頻度が高いかどうか、また、そうであれば、この関連はメタボリックシンドロームに依存したものであるかどうかについて検討した。

対象・方法

2003～2005年までの間に三井記念病院総合検診センターを受診し、上腕－足首間脈波伝播速度（brachial-ankle PWV；baPWV）測定を含む健康評価を受けた952例（男性655例、女性297例）を対象とした。メタボリックシンドロームの診断はNCEP ATPⅢ基準に準じたが、腹囲基準の変わりにbody mass index（BMI）25.0kg/m^{2}以上を用いた。baPWVは、オムロンコーリン社製form ABI/PWVを用いて測定した。症例のbaPWVが最も高い4分位にあった場合（男性：1,721cm/s以上、女性：1,594cm/s以上）、baPWV高値であると定義した。

結果

男女とも、尿酸値が上昇するに従って、baPWV高値の頻度が上昇していた（**図1**）。この関連が、他の交絡因子の補正後でも認められるかどうかについて、動脈硬化の危険因子（年齢、性別、BMI、収縮期血圧、総コレステロール、HDL-コレステロール、中性脂肪、空腹時血糖、喫煙）を共変量としたロジスティック回帰分析により検討した。尿酸値4分位の最低位（男性：5.3mg/dL未満、女性：4.1mg/dL未満）をレファレンスとした場合、尿酸4分位の最高位（男性:7.0mg/dL以上、女性:5.4mg/dL以上）ではbaPWVが高値となるオッズ比が有意に高かった（**表1**）。なお、男性では、レファレンスに比較し、第3の4分位（尿酸値6.1mg/dL以上7.0mg/dL未満）においても、有意に高いオッズ比を有していた。血清尿酸値が上昇するに従い、血清クレアチニン値も上昇していたが、共変量に血清クレアチニン値を追加しても、基本的にはこれらの結果には影響を与えなかった。

対象症例中にメタボリックシンドローム合併が、159例（17%）存在していた。そこで、血清尿酸値とbaPWV高値の関連を、メタボリックシンドロームの有無によるサブグループ別に検討した。性、年齢、性別、BMI、収縮期血圧、総コレステロール、HDL-コレステロール、中性脂肪、空腹時血糖、喫煙を共変量とした多変量ロジスティック回帰分析では、尿酸の4分位の最低位をレファレンスとした場合、最高位の症例のbaPWV高値に対するオッズ比は、メタボリックシンドロームを有しない群において、1.86（95%CI 1.01～1.32）、メタボリックシンドロームを有する群において、4.52（95%CI 1.09～18.72）と、いずれもbaPWV高値のオッズ比が有意に高いことが明らかになった。

考案

本研究では人間ドック受診症例のデータを解析することにより、血清尿酸値とbaPWVによって示される動脈スティフネスの関連を検討した。血清尿酸値が高い場合（男性：6.1mg/dL以上、女性：5.4mg/dL以上）、既知の動脈硬化の危険因子、およびメタボリックシンドロー

図1　血清尿酸値各4分位別とbaPWV高値の割合

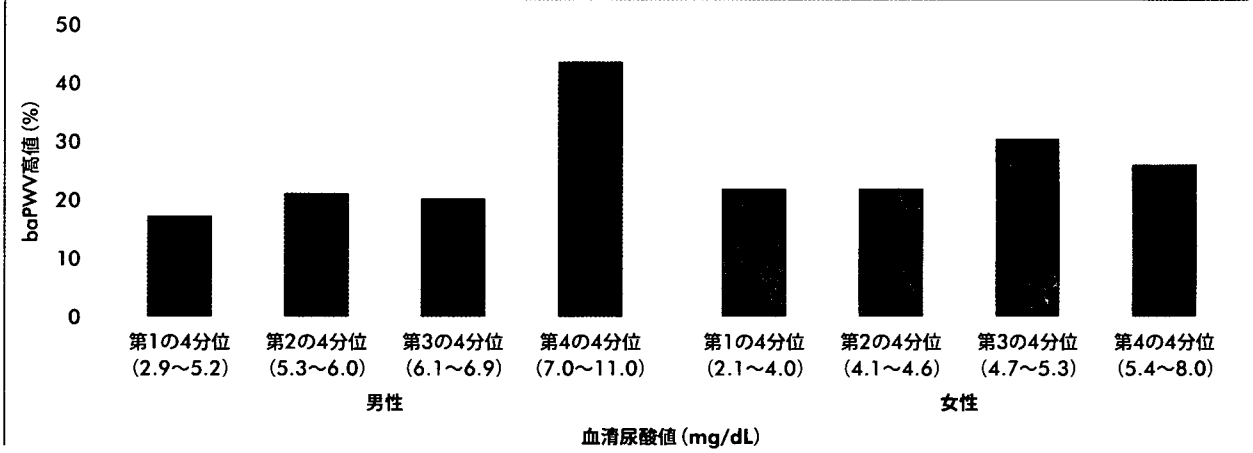

表1　血清尿酸値とbaPWV高値の多変量ロジスティック解析

性、年齢、BMI、収縮期血圧、総コレステロール、HDL-コレステロール、中性脂肪、空腹時血糖、喫煙状況で調整

		男性		女性	
		オッズ比(95% CI)	p値	オッズ比(95% CI)	p値
尿酸	第1の4分位	レファレンス		レファレンス	
	第2の4分位	1.10(0.55～2.20)	0.80	2.80(0.93～8.40)	0.066
	第3の4分位	1.97(1.04～3.75)	<0.05	2.13(0.74～6.19)	0.16
	第4の4分位	2.24(1.10～4.56)	<0.05	2.76(1.01～7.55)	<0.05

ムとは独立に、尿酸値が第1の4分位にある場合と比較してbaPWV高値の頻度が有意に高いことが明らかになった。本研究では男性1,721cm/s以上、女性1,594cm/s以上をbaPWV高値としたが、これらの値が、心血管リスクが増大する可能性があると、以前報告された値(baPWV 1,400cm/s)より高値であることを考えると、本研究で定義されたbaPWV高値は、臨床的にも意味のあるレベルであることが理解される。

結語

人間ドック受診症例において、血清尿酸値が高い症例では、既知の動脈硬化の危険因子、メタボリックシンドロームとは独立に、動脈スティフネスが亢進している可能性が示された。生活習慣改善や、投薬による血清尿酸値の減少が、動脈スティフネス改善に働くかどうか、今後の検討を要する。

Ⅰ．一般演題

メタボリックシンドロームの診断基準と頸動脈肥厚の関連について

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人間ドック受診症例を対象として4つの異なる基準でメタボリックシンドロームを診断し，診断される頻度，頸動脈壁肥厚との関連，という観点から比較検討した．男性ではメタボリックシンドロームと診断する頻度が低い基準ほど，頸動脈壁肥厚との関連が強くかった．男性では，NCEP-ATPIII基準，女性では，IDF基準によるメタボリックシンドロームが最も頸動脈壁肥厚との関連が強かった．

はじめに

血圧や糖・脂質代謝異常が軽度な症例においても，メタボリックシンドロームが存在すると，循環器疾患のリスクが高くなると考えられている．また，メタボリックシンドロームの有無は個々の症例において生活習慣が適切に保たれているかどうかを判断する指標となり得る．わが国においても，高齢化や肥満などを背景に，糖尿病およびその合併症，循環器関連疾患の頻度が増加の一途をたどっており，この概念はますます重要となっていくと考えられる．一方で，わが国の基準も含め，メタボリックシンドロームにはいくつかの診断基準が存在する．本稿では，同じ対象群に対して，4つの異なる基準でメタボリックシンドロームを診断し，診断される頻度，頸動脈壁肥厚との関連，という観点からこれらの診断基準を比較検討した．

1．方法

1）対象

三井記念病院総合健診センターを2005年10月～2006年8月までの間に健康評価のため受診した14,537症例のうち，頸動脈超音波，空腹時血糖/インスリン測定，腹囲測定を施行された1,688例（女性582例，男性1,106例）を対象とした．

2）頸動脈内膜中膜肥厚

頸動脈の内膜中膜厚（IMT）の最大値（max IMT）を男女別に4分位をとり，max IMTが第4の4分位にある場合，「頸動脈肥厚あり」と定義した．これは男性でmax IMTが1.8mm以上，女性で1.5mm以上であった．

3）メタボリックシンドロームの診断基準

診断基準としては，National Cholesterol Education Program Adult Treatment Panel Ⅲ基準（NCEP-ATP Ⅲ）[1]の腹囲をBMI基準に変更したもの（modified NCEP-ATP Ⅲ）[2]，Japan基準[3]，International Diabetes

表 1. 本稿で比較検討したメタボリックシンドロームの診断基準

	NCEP-ATP III基準		modified NCEP-ATP III基準		Japan 基準		IDF 基準	
必須項目	なし		なし		腹囲	男性≧85 cm 女性≧90 cm	腹囲	男性≧85 cm 女性≧90 cm
選択項目	①腹囲	男性>102 cm 女性>88 cm	①BMI	≧25 kg/m²	①血圧	≧130/85 mmHg	①血圧	≧130/85 mmHg
	②血圧	≧130/85 mmHg	②血圧	≧130/85 mmHg	②脂質代謝異常		②中性脂肪	≧150 mg/dl
	③中性脂肪	≧150 mg/dl	③中性脂肪	≧150 mg/dl	中性脂肪	≧150 mg/dl	③空腹時血糖	≧100 mg/dl
	④空腹時血糖	≧110 mg/dl	④空腹時血糖	≧110 mg/dl	HDL-コレ	または	④HDL-コレ	男性<40 mg/dl
	⑤HDL-コレ	男性<40 mg/dl	⑤HDL-コレ	男性<40 mg/dl	ステロール	<40 mg/dl	ステロール	女性<50 mg/dl
	ステロール	女性<50 mg/dl	ステロール	女性<50 mg/dl	③空腹時血糖	≧110/dl		
診断	①~⑤のうち3つ以上		必須項目と①~④のうち2つ以上		必須項目と①~③のうち2つ以上		必須項目と①~③のうち2つ以上	

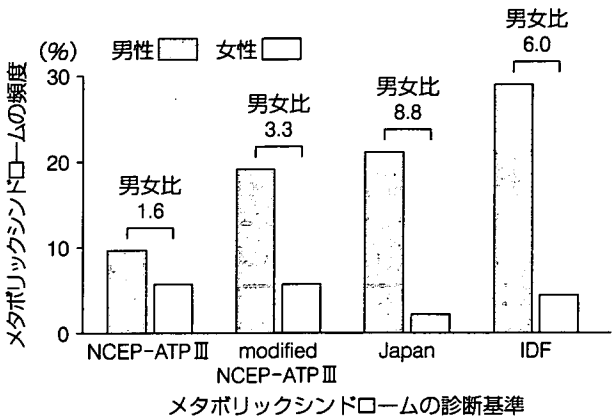

図 1. さまざまな診断基準によるメタボリックシンドローム頻度の差

Federation (IDF) 基準[4]の4種類を用いた（表 1）．なお，IDF 基準では，腹囲のカットオフ値に日本人対象の数値を用いた．

2．各規準で診断されるメタボリックシンドロームの頻度

メタボリックシンドロームの頻度は男性では，NCEP-ATP III<modified NCEP-ATP III<Japan<IDF の順であり，女性では Japan<IDF<modified NCEP-ATP III≒NCEP-ATP III の順であった．また，頻度の男女比をとると，NCEP-ATP III基準で 1.6 と，最も男女間の頻度が近接していたが，わが国の基準では男女比 8.8 とかなりの開きが存在していた（図 1）．

NCEP-ATP III基準と modified NCEP-ATP III基準の差は，肥満の基準として，腹囲を用いているか，BMI を用いているかの差である．BMI と腹囲について検討症例を対象にプロットしてみると，よい相関があることがわかる（図 2）．回帰直線から計算すると，NCEP-ATP III の男性基準 102 cm は，BMI 30.8 kg/m²に，女性の 88 cm は 25.5 kg/m²に相当することがわかる．つまり，女性にとっては，腹囲 88 cm が BMI 25 kg/m²にほぼ相当する値であることが，2つの基準を用いて診断されるメタボリックシンドロームの頻度を近いものにしていると考えられる．ちなみに，本稿の対象群では，Japan 基準における腹囲の男性のカットオフ値 85 cm は BMI 23.3 kg/m²に，女性のカットオフ値 90 cm は BMI 26.4 kg/m²に相当する．

3．メタボリックシンドロームと頸動脈内膜中膜肥厚の関連

つぎに各診断基準で診断されるメタボリックシンドロームがどの程度頸動脈肥厚と関連があるかについて，年齢，喫煙状況を共変量としてロジスティック回帰分析により検討した（図 3）．男性では，いずれの基準で診断されるメタボリックシンドロームも頸動脈内膜中膜肥厚の危険因子となっていることがわかる．オッズ比の順に並べると，IDF<Japan<modified NCEP-ATP III<NCEP-ATP III であり，メタボリックシンドロームと診断される頻度と逆順であることがわかる．女性では，IDF によって診断されるメタボリックシンドローム以外は，頸動脈内膜中膜肥厚との関連は統計的に有意なレベルに達しなかった．女性では IDF 基準に比較して対象集団中で，より頻度が低かった Japan 基準はオッズ比からみても IDF よりも低い値となっていた．

4．他施設からの報告との比較

国内外のいくつかの論文により，メタボリックシンド