vascular Health Study Collaborative Research Group. N Engl J Med 1999;340:14–22.

[21] Okada K, Maeda N, Tatsukawa M, Shimizu C, Sawayama Y, Hayashi J. The influence of lifestyle modification on carotid artery intima-media thickness in a suburban Japanese population. Atherosclerosis 2004;173:329–37.

[22] Tatsukawa M, Sawayama Y, Maeda N, et al. Carotid atherosclerosis and cardiovascular risk factors: a comparison of residents of a rural area of Okinawa with residents of a typical suburban area of Fukuoka, Japan. Atherosclerosis 2004;172:337–43.

[23] Nakajima K, Saito T, Tamura A, et al. Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gels. Clin Chim Acta 1993;223:53–71.

[24] Marcoux C, Tremblay M, Nakajima K, Davignon J, Cohn JS. Characterization of remnant-like particles isolated by immunoaffinity gel from the plasma of type III and type IV hyperlipoproteinemic patients. J Lipid Res 1999;40:636–47.

[25] Zhang B, Tomura H, Kuwabara A, et al. Correlation of high density lipoprotein (HDL)-associated sphingosine 1-phosphate with serum levels of HDL-cholesterol and apolipoproteins. Atherosclerosis 2005;178:199–205.

[26] Zhang B, Noda K, Saku K. Effect of atorvastatin on total lipid profiles assessed by analytical capillary isotachophoresis. Cardiology 2003;99:211–3.

[27] SAS procedure guide version 6. 3rd ed. Cary, NC, USA: SAS Institute Inc.; 1990.

[28] Saku K, Zhang B, Shirai K, et al. Hyperinsulinemic hypoalphalipoproteinemia as a new indicator for coronary heart disease. J Am Coll Cardiol 1999;34:1443–51.

[29] Karpe F, Boquist S, Tang R, et al. Remnant lipoproteins are related to intima-media thickness of the carotid artery independently of LDL cholesterol and plasma triglycerides. J Lipid Res 2001;42:17–21.

[30] Bittolo-Bon G, Cazzolato G. Analytical capillary isotachophoresis of total plasma lipoproteins: a new tool to identify atherogenic low density lipoproteins. J Lipid Res 1999;40:170–7.

[31] Swets JA. ROC analysis applied to the evaluation of medical imaging techniques. Invest Radiol 1979;14:109–21.

Original Article

Both Hepatitis C Virus and *Chlamydia Pneumoniae* Infection are Related to the Progression of Carotid Atherosclerosis in Patients Undergoing Lipids Lowering Therapy

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Abstract Recent experimental and epidemiological findings suggest that infectious agents may play a role in the development and progression of atherosclerosis. We previously reported that *Chlamydia pneumoniae (C. pneumoniae)* infection reduces the effectiveness of lipid-lowering therapy for carotid atherosclerosis and that this microorganism may play a role in the progression of atherosclerosis. In this study, we investigated the possible association between hepatitis C virus (HCV) infection and carotid arteriosclerosis.

A total of 165 asymptomatic hypercholesterolemic patients were randomized to receive probucol (500 mg/day, n=82) or pravastatin (10 mg/day, n=83) and were followed for 2 years. The 2-year change of the maximum common carotid artery intima-media thickness (Max-IMT) was the primary endpoint, while the Max-IMT and the incidence of major cardiovascular events were secondary endpoint. All serum samples were tested for antibody to HCV (anti-HCV) by enzyme-linked immunosorbent assay (ELISA), and all anti-HCV-positive samples were assayed for HCV RNA. Patients without HCV infection (n=25) showed a significant reduction of Max-IMT (−10.9%) ($p<0.0001$), while a small decrease of Max-IMT was noted in the patients with HCV infection (n=25) (−0.3%). Significant differences in the reduction of serum total cholesterol and LDL cholesterol were found between patients with and without HCV infection (both $p<0.0001$). No significant difference in therapeutic effect was noted between the probucol and the pravastatin groups. After adjustment for confounding risk factors, both *C. pneumoniae* infection and anti-HCV positivity were associated with a greater risk of an increase in Max-IMT (8.5635 [1.3738-15.7532], $p<0.05$, 9.5040 [0.2886-18.7194], $p<0.05$, respectively). These findings suggest that both chronic HCV infection and *C. pneumoniae* infection can reduce the effectiveness of lipid-lowering therapy for carotid atherosclerosis, and that the HCV may play a role in the progression of atherosclerosis in HCV infected patients.

Key words: Hepatitis C virus, probucol, *Chlamydia pneumoniae*, pravastatin, intima-media thickness, carotid atherosclerosis, cardiovascular disease

INTRODUCTION

Atherosclerosis is a highly prevalent disease, and it is currently the single greatest source of morbidity and mortality in developed societies. Many risk factors are involved in the development of atherosclerosis, which manifests as coronary artery disease (CAD) and myocardial infarction,

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including hyperlipidemia, hypertension, smoking, and diabetes mellitus[1]. Infection has also been suggested to be associated with an increased risk of atherosclerosis. Infection induces an inflammatory response and the mechanism of atherosclerosis has an inflammatory component. Currently, it is unclear whether the general inflammatory response to an infectious agent has an important role in the development of atherosclerosis or whether there are some specific atherogenic microorganisms[2]. Certain microorganisms have been suggested to directly cause or exacerbate atherosclerosis, including *Chlamydia pneumoniae (C. pneumoniae)*, Cytomegalovirus, Herpes simplex virus, and *Helicobacter pylori*[3)~7)]. Our previous study revealed a significant reduction in the rate of intima-media thickness (IMT) progression in *C. pneumoniae*-negative patients versus no such significant reduction in *C. pneumoniae*-positive patients, even though both groups of patients had significant reductions of total cholesterol and LDL cholesterol. These results suggested that *C. pneumoniae* infection could influence the carotid artery IMT[8].

The various hepatitis viruses primarily target the liver and cause a characteristic inflammatory process, with the hepatitis A virus (HAV), hepatitis B virus (HBV), and hepatitis C virus (HCV) having been investigated for a possible atherogenic effect. HAV was reported to be associated with coronary artery disease[9], but enterically transmitted hepatitis A infection is a self-limiting disease that does not progress to chronic inflammation, whereas hepatitis B or C infection has the potential for chronicity. The results of two previous studies[10)11)] have suggested that hepatitis B and C infection may be independent risk factors for carotid atherosclerosis. However, such findings have not necessarily been confirmed[12)] and one study[13)] has even shown the conflicting result that hepatitis virus infection is protective against atherosclerosis.

Against this background, the aim of the present study was to investigate the association between hepatitis virus infection and changes of the carotid artery IMT in patients on lipid-lowering therapy for carotid atherosclerosis. To our knowledge, this is the first investigation of the effectiveness of lipid-lowering therapy for carotid atherosclerosis in relation to not only *C. pneumoniae* infection, but also HCV infection.

METHODS

Patient selection and study protocol

The study design and the baseline characteristics of the patients have already been described elsewhere[14]. Briefly, between February 1996 and February 2000, asymptomatic hypercholesterolemic men and women aged 30-89 years who met the following criteria were enrolled in the present study. Patients were either untreated or were on therapy with a total cholesterol level above 220 mg/dl. Of the 246 patients screened for eligibility, 165 were recruited and were included in the intention-to-treat population. Patients were randomly assigned to either a probucol group (n=82, aged 41 to 80 years) who received probucol at 500 mg twice daily after meals or a pravastatin group (n=83, aged 41 to 89 years) who received pravastatin at 10 mg once daily after the evening meal. Randomization was performed by the minimization method with control of the following four factors : total cholesterol, age, sex, and IMT. Monitoring was done at 2 weeks

after enrollment and every 4 weeks thereafter. At each review, a brief physical examination was performed and the number of tablets was counted to assess compliance. In both groups, lipids, lipoproteins, and others laboratory parameters (to confirm safety) were also measured at each review. Thirty-four of the 165 patients (21%) did not complete the study. Written informed consent was obtained from each patient, and the trial was approved by the Ethics Committee of Kyushu University Hospital.

Exclusion criteria included a serum triglyceride level >350 mg/dl, uncontrolled heart failure, recent myocardial infarction (≤6 months previously), severe or unstable angina pectoris, hypothyroidism/hyperthyroidism or other endocrine diseases, secondary hyperlipidemia, uncontrolled diabetes mellitus, uncontrolled hypertension, heavy drinking, obese patients on weight reduction programs, diseases that might interfere with drug absorption, any other severe illnesses, and treatment with certain drugs including corticosteroids, androgens, other lipid-lowering agents, or antacids containing aluminum salts.

The ultrasound procedure used for carotid IMT measurement and its reproducibility have already been described elsewhere[15]. In brief, ultrasonography was done with the patient in the supine position using an Aloka SSD-2000 (Aloka, Tokyo, Japan) with a 7.5 MHz transducer. In both the right and left common carotid arteries, the IMT of the far wall of the vessel was measured at 2, 2.5, and 3 cm proximal to the carotid bifurcation. The IMT was defined as the distance between two echogenic lines separated by a hypoechoic or anechoic space, with the outer line corresponding to the medial-adventitial border and the inner line representing the luminal-intimal border. The mean IMT was calculated as the average value of the IMT measurements obtained from a total of 6 sites in the carotid arteries (3 on the left and 3 on the right). Stenosis was defined as plaque (any site where the IMT was (1.10 mm) that occupied more than half of the luminal circumference of the artery on a transverse scan.

Laboratory parameters

Blood samples were collected between 8 and 9 AM after a 12-hour fast. Serum cholesterol and triglycerides were measured enzymatically. HDL cholesterol was measured by the heparin Ca method in the supernatant obtained after precipitation of apolipoprotein B-containing lipoproteins, while LDL cholesterol was calculated using the Friedewald formula[16]. All assays were done on the day of blood collection except for measurement of Lp (a), for which blood was stored for a maximum of 3 days at -4(C. Antibody to HCV (anti-HCV) and antibody to HAV (anti-HAV) were measured by commercial enzyme-linked immunosorbent assay (ELISA) methods, while hepatitis B surface antigen (HBsAg) was determined by a latex agglutination immunoassay using stored frozen samples. Then the HCV RNA was assayed by polymerase chain reaction.

Statistical analysis

Data were recorded on standard forms and then entered into a database. Results are expressed as percentages or as the mean ±standard deviation (SD). Differences between categorical variables were compared using Yates' corrected chi-square test, while comparison of continuous variables was done by the Student's t-test.

The endpoint of this study was the change

of the Max-IMT (mm) after 24 months. Multiple linear regression analysis was done to detect factors that influenced the rate of change of Max-IMT from among HAV seropositivity, HBV seropositivity, HCV seropositivity, age, sex, smoking history, diabetes, and hypertension. Variables were selected for entry into the model by the backward stepwise method. A probability value of less than 0.05 was considered to indicate statistical significance in all analyses. Data were analyzed on an intention-to-treat basis.

RESULTS

Baseline characteristics and comparability

The mean age of the patients was 65.7 years and 24% were men. Average systolic blood pressure and diastolic blood pressure were 131 and 76 mm Hg, respectively. Of the 165 patients, 46% were recent or former smokers, 38% had a history of hypertension, and 17% had diabetes mellitus. Baseline serum total cholesterol and LDL cholesterol levels were 251 mg/dL and 164 mg/dL, respectively. The HDL cholesterol level was 57 mg/dL, while the serum triglyceride level was 150 mg/dL. The Max-IMT was 1.59 mm. There were no statistically significant differences between the two groups for any of these baseline characteristics. Anti-HAV was detected in 67.5%, HBsAg was positive in 2.4%, and anti-HCV was positive in 15.2% of the patients. HCV-RNA was detected in all of the anti-HCV positive patients.

Percent reduction of total cholesterol after 24 months stratified by HAV, HBV, HCV, and *C. pneumoniae* status

There was a significant reduction of the serum total cholesterol level by 21.7% between baseline (247.2 ± 24.6 mg/dl) and 24 months of therapy (192.5 ± 25.9 mg/dl) in the patients without HAV infection and a decrease of 20.8% in the infected patients (both $p<0.001$, Student's t-test). Significant reduction of the serum total cholesterol level by 21.3% was also seen between baseline (248.9 ± 24.3 mg/dl) and 24 months of therapy (195.9 ± 30.3 mg/dl) in the patients without HBV infection and there was a decrease of 10.8% in the infected patients ($p<0.05$ and $p<0.001$, respectively; Student's t-test). Moreover, a significant reduction of serum total cholesterol by 21.1% occurred between baseline (246.8 ± 23.7 mg/dl) and 24 months (194.1 ± 25.6 mg/dl) in the patients without HCV infection, as well as a decrease of 21.1% in the infected patients (both $p<0.001$, Student's t-test). There were no significant differences between probucol and pravastatin therapy with respect to the reduction of total cholesterol in patients with or without HAV, HBV, and HCV infection after 24 months of treatment (Table 1).

Percent reduction of LDL cholesterol after 24 months stratified by HAV, HBV, HCV, and *C. pneumoniae* status

Significant reduction of the serum LDL cholesterol level by 26.9% was found between baseline (154.0 ± 30.7 mg/dl) and 24 months of therapy (110.0 ± 27.1 mg/dl) in the patients without HAV infection, while the decrease was 27.6% in the infected patients (both $p<0.001$, Student's t-test). Significant reduction of the LDL cholesterol level by 27.4% was also seen between baseline (157.2 ± 27.7 mg/dl) and 24 months (113.6 ± 31.4 mg/dl) in the patients without HBV infection and there was a decline of 27.3% in the infected patients ($p < 0.05$ and $p < 0.001$, respectively;

Table 1 Changes of total cholesterol after 24 months of treatment stratified by HAV, HBV, and HCV status

	No.	Total cholesterol (mg/dl, mean ± SD)		P value (Student's t-test)	Percent change (mg/dl, mean ± SD)	P value (paired t-test)
		before	after 24 months			
anti-HAV						
+	113	248.4+23.3	196.5+30.4	<0.0001	20.8+10.8	NS
−	52	247.2+24.6	192.5+25.9	<0.0001	21.7+10.6	
HBs Ag						
+	4	244.0+12.7	217.8+18.0	<0.05	10.8+4.6	NS
−	161	248.0+87.5	194.7+29.1	<0.0001	21.3+10.7	
anti-HCV						
+	25	242.8+18.8	191.3+20.9	<0.0001	20.8+9.8	NS
−	140	248.9+24.3	195.9+30.3	<0.0001	21.1+10.9	
ant-*C. pneumoniae*-IgA and/or IgG #						
+	115	247+22	195+29	<0.0001	21	NS
−	50	249+27	197+30	<0.0001	21	
Treatment						
Probucol	82	249.4+23.6	196.5+32.8	<0.0001	21.1+12.0	NS
Pravastatin	83	246.8+23.7	194.1+25.6	<0.0001	21.1+9.5	

Data quoted from Sawayama et al. Atherosclerosis 2003; 171 (2): 281-285.

Table 2 Changes of LDL cholesterol after 24 months of treatment stratified by HAV, HBV, and HCV status

	No.	Total cholesterol (mg/dl, mean ± SD)		P value (Student's t-test)	Percent change (mg/dl, mean ± SD)	P value (paired t-test)
		before	after 24 months			
anti-HAV						
+	113	158.3+26.1	114.8+33.4	<0.0001	27.6+19.2	NS
−	52	154.0+30.7	111.0+27.1	<0.0001	26.9+15.9	
HBs Ag						
+	4	146.2+25.7	112.4+39.5	<0.0001	24.1+21.6	NS
−	161	157.2+27.7	113.6+31.4	<0.0001	27.4+18.2	
anti-HCV						
+	25	154.9+25.2	118.0+23.9	<0.0001	22.6+16.2	NS
−	140	157.3+28.1	112.8+32.6	<0.0001	28.2+18.4	
ant-*C. pneumoniae*-IgA and/or IgG #						
+	115	157+27	112+32	<0.0001	28	NS
−	50	159+29	117+30		26	
Treatment						
Probucol	82	162.7+24.8	125.6+31.7	<0.0001	22.3+18.6	<0.0001
Pravastatin	83	151.9+29.0	103.2+27.5	<0.0001	31.8+16.7	

Data quoted from Sawayama et al. Atherosclerosis 2003; 171 (2): 281-285.

Student's t-test). Moreover, a significant reduction of LDL cholesterol by 28.2% occurred between baseline (157.3 ± 28.1 mg/dl) and 24 months (154.9 ± 25.2 mg/dl) in the patients without HCV infection, while the decrease was 22.6% in the infected patients (both $p<0.001$, Student's t-test). A significantly greater reduction of LDL cholesterol levels was achieved by pravastatin than probucol in patients with HAV, HBV, HCV, and *C. pneumoniae* infection after 24 months of treatment (Table 2).

Percent reduction of Max-IMT after 24 months stratified by HAV, HBV, HCV, and *C. pneumoniae* status

A significant decrease of Max-IMT by 9.7% was found between baseline (1.28 ± 0.57 mm) and 24 months of therapy (1.10 ± 0.45 mm) in the patients without HAV

Table 3 Changes of Max-IMT after 24 months of treatment stratified by HAV, HBV, and HCV status

	No.	Total cholesterol (mg/dl, mean ± SD)		P value (Student's t-test)	Percent change (mg/dl, mean ± SD)	P value (paired t-test)
		before	after 24 months			
anti-HAV						
+	113	1.54+0.88	1.36+0.78	<0.0001	9.1+21.5	NS
−	52	1.28+0.57	1.10+0.45	<0.0001	9.7+23.7	
HBs Ag						
+	4	1.28+0.15	1.13+0.15	<0.0577	11.7+7.9	NS
−	161	1.46+0.81	1.28+0.71	<0.0001	9.2+22.4	
anti-HCV						
+	25	1.51+0.91	1.44+0.83	NS	0.3+22.5	<0.05
−	140	1.45+0.78	1.25+0.67	<0.0001	10.9+121.7	
ant-*C. pneumoniae*-IgA and/or IgG #						
+	115	1.27+0.62*	1.19+0.64*	NS	6	<0.01
−	50	1.33+0.60*	1.08+0.50*	<0.01	19	
Treatment						
Probucol	82	1.59+0.90	1.35+0.67	<0.0001	9.6+24.0	NS
Pravastatin	83	1.35+0.70	1.21+0.73	<0.0001	9.0+20.5	

Data quoted from Sawayama et al. Atherosclerosis 2003; 171 (2): 281-285.
* IMT is the mean IMT value.

infection and there was a decrease of 9.1% in the infected patients (both $p<0.001$, Student's t-test). A reduction of Max-IMT by 9.2% occurred between baseline (1.46 ± 0.81 mm) and 24 months (1.28 ± 0.71 mm) in the patients without HBV infection while there was a decrease of 11.7% in the infected patients ($p<0.001$ and $p=0.057$, respectively; Student's t-test). In contrast, a reduction of Max-IMT by 10.9% was seen between baseline (1.45 ± 0.81 mm) and 24 months (1.25 ± 0.67 mm) in the patients without HCV infection versus a decrease of only 0.3% in the infected patients ($p<0.001$ and $p=0.4104$, respectively; Student's t-test). Although the reduction of IMT was 9.2% versus 11.7% in the patients with and without HBV infection, respectively, showing no significant difference, there was a significant difference of the change of Max-IMT between the patients with and without HCV infection (0.3% versus 10.9%, respectively). There were no significant differences between probucol and pravastatin therapy with regard to the reduction of Max-IMT after 24 months in relation to HAV, HBV, HCV, and *C. pneumoniae* infection status (Table 3).

Multiple linear regression analysis

Backward stepwise multiple linear regression analysis revealed that not only *C. pneumoniae* infection, but also HCV infection showed a strong independent association with a smaller reduction of IMT ($p=0.0276$). There was no significant association between the change of IMT and any of the other variables investigated (Table 4).

Serious adverse events

Among the 25 patients with HCV infection, two had a major cardiovascular event (2 deaths from coronary heart disease) compared with 0 of the 140 uninfected patients. Of the four patients in this study who died, 3 had HCV infection. There was a lower incidence of death in the patients without HCV infection than in the infected patients, but the difference was not significant. There were no significant differences of adverse events between probucol and pravastatin therapy.

Table 4 Multiple linear regression analysis of factors influencing Max-IMT

Fixed	Difference	95% CI		P value
anti-HAV	−1.5412	−9.1996	6.1172	0.6897
HBs Ag	−3.0118	−25.3981	19.3745	0.7917
anti-HCV	9.0000	−0.6367	18.6367	0.0692
C. pneumonia-IgA	−0.6227	−10.7632	9.5178	0.9205
C. pneumonia-IgG	1.0917	−8.5300	10.7134	0.8234
C. pneumonia-IgA and/or IgG	7.5701	−6.0267	21.1669	0.2771
Age >75 years	5.3447	−3.1304	13.8198	0.2180
Male sex	−4.7624	−13.6453	4.1205	0.2959
Smoking	−4.1964	−11.9445	3.5517	0.2895
Hypertension	5.2711	−2.0015	12.5437	0.1573
Diabetes	6.2849	−3.4671	16.0369	0.2078
Backward stepwise	Difference	95% CI		P value
anti-HCV	9.5040	0.2886	18.7194	0.0448
C. pneumonia-IgA and/or IgG	8.5635	1.3738	15.7532	0.0208

DISCUSSION

The present study adds new information to the growing pool of data regarding lipid-lowering therapy for carotid atherosclerosis in patients with HCV infection. A significant reduction of Max-IMT was found in HCV-negative patients, while no significant reduction was seen in the HCV-positive patients, even though both groups of patients showed significant improvement of total cholesterol and LDL cholesterol. These results suggest that HCV infection influences the carotid artery IMT, as does *C. pneumoniae* infection. In contrast, there was no association between HAV or HBV infection and the changes of Max-IMT.

Our results are in concordance with those of another Japanese study[11] performed on company employees undergoing regular health checks that found a relationship between HCV positivity and carotid atherosclerotic plaque or IMT. We previously reported that the prevalence of HCV infection was 3.3-19.7% in northern Kyushu Island, including the area surveyed in the present study[17)~19)]. In general, the clinical course of chronic HCV infection is characterized by a series of exacerbations and remissions, but it may eventually progress to hepatic decompensation and the development of cirrhosis. Usually, the onset of cirrhosis appears to be associated with a decreased risk of atherosclerosis, which may be explained by the decreased production of clotting factors and the reduction of certain conventional risk factors such as total cholesterol and lipoprotein (a)[20]. Therefore, the positive correlation between HCV infection and carotid atherosclerosis observed in the present study was rather unexpected. Because serum total cholesterol and LDL cholesterol levels were not significantly different between our HCV-positive and HCV-negative subjects, it seems that liver dysfunction was not severe in the majority of the HCV-positive patients. Kiechl et al.[21] found no significant association between chronic hepatitis and carotid plaque, although whether their subjects had HBV or HCV infection was not specified. These different results may have been obtained because they analyzed patients with chronic active hepatitis, whereas most of the subjects in our study did not have been active.

Several previous studies have suggested that certain microorganisms may contribute

to the pathogenesis of atherosclerosis, including *C. pneumoniae*[3] and *Helicobacter pylori*[22]. Such bacteria may cause vascular injury by direct colonization[23] or by activation of a systemic inflammatory response, which may play a role in the progression and destabilization of atherosclerotic plaques. In support of this view, we found a higher incidence of death in patients with *C. pneumoniae* infection than in the uninfected patients, but the difference was not statistically significant[8]. A new study with a longer observation period will be required to determine the precise relationship between *C. pneumoniae* infection and CAD, as well as why C. pneumoniae infection is associated with a resistance of carotid atherosclerosis to lipid-lowering therapy.

The following possible mechanisms may explain the relationship between HCV infection and atherosclerosis. First, HCV infection is occasionally associated with vasculitis[24]. Second, chronic HCV infection may be associated with an increase of oxidative stress[25]. Third, HCV infection can produce elements of the metabolic syndrome by inducing insulin resistance[26], which may accelerate atherogenesis. Finally, chronic HCV infection may stimulate a systemic inflammatory response. Another study with a longer observation period will be required to determine the precise relationship between HCV infection and atherosclerosis, as well as why HCV infection was associated with the effect of lipid-lowering therapy on carotid atherosclerosis.

Evidence from epidemiological and clinical studies has shown that LDL cholesterol is very important in the development of atherosclerosis and that reducing the LDL cholesterol level can also reduce the risk of CAD. We previously reported that active treatment may not only have a lipid-lowering effect but may also stabilize plaque and in the same study demonstrated a lower incidence of cardiac events in the treated group than in the control group[3]. In the present study, we found a significant reduction of the serum total and LDL cholesterol levels in both HCV-infected and uninfected patients. Although total and LDL cholesterol levels were reduced in the HCV-infected patients by active treatment, the carotid IMT was not improved as occurred in the uninfected patients. The above finding suggests that HCV infection is a risk factors for progression of atherosclerosis. If it possible that the organism may be an innocent bystander in atherosclerotic tissue, rather than an inciter of chronic inflammation. Determining the exact nature of the association between HCV and atherosclerosis is important. The above suggests that HCV was found to be a causative factor or to significantly contribute to the progression of atherosclerosis, so it might be improved by the interferon therapy which is able to eliminates HCV RNA in patients with chronic HCV infection[27][28].

The present study had some limitations. First, the subjects were only defined by their serological parameters and not by histology. However, histological examination is more suitable for a clinical setting than for population-based studies. Second, the duration of exposure to viral infection could not be estimated because the detection of antibodies was usually incidental. Thus, the duration of inflammation may have been too short and the infection too mild to detect any pro-atherosclerotic effect in some of the subjects. Although care was taken to avoid potential biases, it is well known that prospective studies are often

unable to confirm the associations detected by case-control studies. Therefore, a future prospective study is needed to confirm our findings.

In conclusion, our observations suggest that both HCV infection and *C. pneumoniae* infection reduce the efficacy of lipid-lowering therapy for carotid atherosclerosis, and that HCV may play a role in the development/progression of atherosclerosis.

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REFERENCES

1) Ross R: Atherosclerosis: an inflammatory disease. N Engl J Med 340: 115-126, 1999.
2) Muhlestein JB: Chronic infection and coronary heart disease. Med Clin North Am 84: 123-148, 2000.
3) Saikku P, Leinonen M, Mattila K, Ekman MR, Nieminen MS, Makela PH, Huttunen JK and Valtonen V: Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet 2: 983-986, 1988.
4) Adam E, Melnick JL, Probtsfield JL, Petrie BL, Burek J,Bailey KR, McCollum CH and DeBakey ME: High levels of cytomegalovirus antibody in patients requiring vascular surgery for atherosclerosis. Lancet 2: 291-293, 1987.
5) Horvath R, Cerny J, Benedik J Jr, Hokl J, Jelinkova I and Benedik J: The possible role of human cytomegalovirus (CMV) in the origin of atherosclerosis. J Clin Virol 16: 17-24, 2000.
6) Yamashiroya HM, Ghosh L, Yang R and Robertson AL Jr: Herpesviridae in the coronary arteries and aorta of young trauma victims. Am J Pathol 130: 71-79, 1988.
7) Mendall MA, Goggin PM, Molineaux N, Levy J, Toosy T, Strachan D, Camm AJ and Northfield TC: Relation of Helicobacter pylori infection and coronary heart disease. Br Heart J 71: 437-439, 1994.
8) Sawayama Y, Tatsukawa M, Okada K, Maeda N, Shimizu C, Kikuchi K and Hayashi J: Association of Chlamydia pneumoniae antibody with the cholesterol-lowering effect of statins. Atherosclerosis 171 (2): 281-285, 2003
9) Zhu J, Quyyumi AA, Norman JE, Costello R, Csako G and Epstein SE: The possible role of hepatitis A virus in the pathogenesis of atherosclerosis. J Infect Dis 182: 1583-1587, 2000.
10) Ishizaka N, Ishizaka Y, Takahashi E, Toda Ei E, Hashimoto H, Ohno M, Nagai R and Yamakado M: Increased prevalence of carotid atherosclerosis in hepatitis B virus carriers. Circulation 105: 1028-1030, 2002.
11) Ishizaka N, Ishizaka Y, Takahashi E, Tooda E, Hashimoto H, Nagai R and Yamakado M: Association between hepatitis C virus seropositivity, carotid artery plaque, and intima-media thickening. Lancet 359: 133-135, 2002.
12) Kiechl S, Egger G, Mayr M, Wiedermann CJ, Bonora E, Oberhollenzer F, Muggeo M, Xu Q, Wick G, Poewe W and Willeit J: Chronic infections and the risk of carotid atherosclerosis: prospective results from a large population study. Circulation 103: 1064-1070, 2001.
13) Bilora F, Rinaldi R, Boccioletti V, Petrobelli F and Girolami A: Chronic viral hepatitis: a prospective factor against athersosclerosis. A study with echo-color Doppler of the carotid and femoral arteries and the abdominal aorta. Gastroenterol Clin Biol 26: 1001-1004, 2002.
14) Sawayama Y, Nabeshima S, Taniai H, Furusyo N, Kashiwagi S and Hayashi J: Effects of probucol and pravastatin on common carotid atherosclerosis in patients with asymptomatic hypercholesterolemia: Fukuoka Atherosclerosis Trial (FAST). J Am Coll Cardiol 39 (4): 600-616, 2002.
15) Werba JP, Safa O, Gianfranceschi G, Michelagnoli S, Sirtori CR and Franceschini G: Plasma triglycerides and lipoprotein (a): inverse relationship in a

hyperlipidemic Italian population. Atherosclerosis 101 (2): 203-211, 1993.
16) Ramirez JA: Isolation of Chlamydia pneumoniae from the coronary artery of a patient with coronary atherosclerosis. The Chlamydia pneumoniae/Atherosclerosis Study Group. Ann Intern Med 125 (12): 979-982, 1996.
17) Hayashi J, Kishihara Y, Yamaji K, Yoshimura E, Kawakami Y, Akazawa K and Kashiwagi S: Transmission of hepatitis C virus by health care workers in a rural area of Japan. Am J Gastroenterol 90 (5): 794-799, 1995.
18) Hayashi J, Yoshimura E, Nabeshima A, Kishihara Y, Ikematsu H, Hirata M, Maeda Y and Kashiwagi S: Seroepidemiology of hepatitis C virus infection in hemodialysis patients and the general population in Fukuoka and Okinawa, Japan. J Gastroenterol 29 (3): 276-281, 1994.
19) Hayashi J, Furusyo N, Sawayama Y, Kishihara Y, Kawakami Y, Ariyama I, Etoh Y and Kashiwagi S: Hepatitis G virus in the general population and in patients on hemodialysis. Dig Dis Sci 43: 2143-2148, 1998.
20) Marchesini G, Ronchi M, Forlani G, Bugianesi E, Bianchi G, Fabbri A, Zoli M and Melchionda N: Cardiovascular disease in cirrhosis: a point-prevalence study in relation to glucose tolerance. Am J Gastroenterol 94: 655-662, 1999.
21) Kiechl S, Egger G, Mayr M, Wiedermann CJ, Bonora E, Oberhollenzer F, Muggeo M, Xu Q, Wick G, Poewe W and Willeit J: Chronic infections and the risk of carotid atherosclerosis: prospective results from a large population study. Circulation 103: 1064-1070, 2001.
22) Mendall MA, Goggin PM, Molineaux N, Levy J, Toosy T, Strachan D, Camm AJ and Northfield TC: Relation of Helicobacter pylori infection and coronary heart disease. Br Heart J 71: 437-443, 1994.
23) Chiu B, Viira E, Tucker W and Fong IW: Chlamydia pneumoniae, cytomegalovirus, and herpes simplex virus in atherosclerosis of the carotid artery. Circulation 96: 2144-2148, 1997.
24) Guillevin L, Lhote F and Gherardi R: The spectrum and treatment of virus-associated vasculitides. Curr Opin Rheumatol 9: 31-36, 1997.
25) Sumida Y, Nakashima T, Yoh T, Kakisaka Y, Nakajima Y, Ishikawa H, Mitsuyoshi H, Okanoue T, Nakamura H and Yodoi J: Serum thioredoxin elucidates the significance of serum ferritin as a marker of oxidative stress in chronic liver diseases. Liver 21: 295-299, 2001.
26) Koike K: Hepatitis C virus infection can present with metabolic disease by inducing insulin resistance. Intervirology 49 (1-2): 51-57, 2006.
27) Furusyo N, Hayashi J, Ohmiya M, Sawayama Y, Kawakami Y, Ariyama I, Kinukawa N and Kashiwagi S: Differences between interferon-alpha and -beta treatment for patients with chronic hepatitis C virus infection. Dig Dis Sci. 44 (3): 608-617, 1999.
28) Hayashi J, Ohmiya M, Kishihara Y, Tani Y, Kinukawa N, Ikematsu H and Kashiwagi S: A statistical analysis of predictive factors of response to human lymphoblastoid interferon in patients with chronic hepatitis C. Am J Gastroenterol. 89 (12): 2151-2156, 1994.
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(和文抄録)

脂質低下療法による動脈硬化症の進展抑制における C型肝炎ウイルス及肺炎クラミジア感染の影響

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【目的】 慢性感染症は冠動脈疾患の危険因子の一つとされており,私どもは以前肺炎クラミジア感染に関して報告した. 今回, C型肝炎ウイルス感染が独立して脂質低下療法による動脈硬化症の進展抑制に影響を及ぼすかどうかについて検討した.

【方法】 対象は高コレステロール (TC) 血症患者165例 (30歳～89歳・平均年齢64歳, 男性45例, 女性120例)を対象とし, 抗高脂血症剤(プロブコールあるいはプラバスタチン) を投与し, 血清脂質の測定およびBモード超音波法による総頚動脈の内膜中膜複合体厚 (IMT) の測定を施行し, 2年間経過観察した. HA抗体(ELISA法), HBs抗原(ELISA法), HCV RNA(PCR法), 肺炎クラミジア-IgA, IgG抗体 (ELISA法) で測定した.

【結果】 血清脂質値は, 脂質低下療法にてHAV, HBV及びHCVの感染の有無にかかわらず, いずれも有意な減少を認めた. Max-IMT値の減少率は, HAV及びHBVの感染の有無にかかわらず有意差はみられなかったが, HCV感染陰性の場合は, 感染陽性の場合より脂質低下療法による動脈硬化の進展抑制効果が有意に認められた($P<0.05$). さらに多変量解析にて, Max-IMT変化率に寄与する因子を検討したところ,肺炎クラミジア感染及びHCV感染は治療効果の独立した負の因子であった.またプロブコールとプラバスタチンの間では感染の有無および治療効果に差はみられなかった.

【考察】 肺炎クラミジア感染だけでなく, HCVの持続感染も,脂質低下療法による動脈硬化症の進展抑制効果を妨げる可能性が示唆された.

Original Article

The Impact of Peripheral Arterial Disease and Acute Ischemic Stroke

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Abstract Peripheral arterial disease (PAD) is associated with coronary artery disease (CAD) and stroke, but data on the relationship between PAD and acute ischemic stroke are lacking. Therefore, we investigated this relationship. A total of 101 patients were enrolled on admission to Harasanshin General Hospital (Fukuoka, Japan) with their first ischemic stroke. All 101 patients underwent cranial CT and/or brain magnetic resonance imaging, duplex ultrasonography of the extracranial carotid arteries, and transthoracic echocardiography.

The subjects were aged 41 to 92 years. PAD was present in 81/101 patients (80.2%), including 57/73 (78.1%) with small artery occlusion, 11/13 (84.6%) with large artery occlusion, and 13/15 (86.7%) with cardiogenic embolism. In 42 of these 81 patients (51.9%), PAD was asymptomatic. Serum apoprotein A1 levels were significantly higher and the intima-media thickness was significantly greater in the patients with PAD than in those without PAD. The modified Rankin scale score was significantly higher on admission in patients with PAD than in those without PAD. Stepwise logistic regression analysis revealed that the apoprotein A1 level and the modified Rankin scale score on admission were strongly associated with the occurrence of stroke in patients with PAD.

Our results suggest that PAD is frequently associated with acute ischemic stroke. It may be important to perform screening for PAD in patients who have suffered an ischemic stroke.

Key words: peripheral arterial disease, ischemic stroke, stroke subtypes, carotid atherosclerosis

INTRODUCTION

Atherosclerosis is a highly prevalent disease, and is currently the greatest cause of morbidity and mortality in developed societies. Many risk factors are involved in the occurrence of atherosclerosis, which manifests as coronary artery disease (CAD) and myocardial infarction (MI), including hyperlipidemia, hypertension, smoking, and diabetes mellitus[1]. It is also known that peripheral arterial disease (PAD) is associated with CAD and stoke, but data on the relationship between peripheral arterial disease and acute ischemic stroke are lacking. The pulse wave velocity can be used as an indicator of arterial stiffness[2][3], and it is regarded as a marker of vascular damage[4][5]. An instrument was recently developed that can measure the brachial-ankle

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pulse wave velocity (baPWV) by the volume-rendering method. Yamashina et al. have reported a high validity and reproducibility of baPWV measurements, suggesting that this parameter may be an acceptable indicator of vascular damage and may be suitable for screening large populations to detect vascular disease[6].

Recently, arteriosclerotic disease has been increasing in Japan as the population ages and the lifestyle becomes more westernized. The prevalence of arteriosclerosis is therefore anticipated to increase in Japan and the prevalence of PAD is also expected to increase. However, there have been few epidemiological studies on PAD in Japan and even fewer studies on the prevalence of PAD among stroke patients. This is partly because early PAD is asymptomatic and therefore difficult to diagnose and also because distinguishing PAD from other conditions, such as spinal cord disease, is difficult even after PAD symptoms like nocturnal leg pain become evident. PAD not only interferes with daily activities and reduces the quality of life in stroke patients, but also worsens their survival. PAD is associated with the occurrence of coronary artery disease and cerebrovascular disease[7] Therefore, PAD should be detected and treated as early as possible.

We performed the present prospective study to investigate whether PAD was an independent risk factor for acute ischemic stroke in Japanese patients.

METHODS

Subjects

All of the patients with acute ischemic stroke admitted to the Division of General Medicine at Harasanshin General Hospital (Fukuoka, Japan) during the period from August 1, 2003 to July 31, 2004 were eligible for the present study. As a result, a total of 101 patients who suffered their first ischemic stroke were registered for this study after meeting the following criteria: (a) first ischemic stroke, (b) admission to hospital for treatment, and (c) admission within 72 hours of the onset.

Categorization of Stroke

Stroke was defined according to World Health Organization criteria[8]. Cerebral infarction was diagnosed on the basis of the initial CT and MRI data. All patients underwent ultrasonography of the neck and intracranial arteries. The carotid arteries were assessed by color flow B-mode Doppler ultrasound (SONOS 5500, PHILIP) according to the standard method[9][10]. The vertebrobasilar system was evaluated by radionucleotide angiography to determine the presence/absence of atherosclerotic lesions. Patients without clinical or imaging evidence of atherosclerosis who had atrial fibrillation and/or echocardiographic findings suggestive of possible cardiogenic embolism were classified as having thromboembolic stroke. The other patients were diagnosed as having large artery stroke if there was >50% stenosis of the extracranial carotid artery or an intracranial artery, or as having small artery occlusion if they had a clinical lacunar syndrome associated with appropriate CT changes or a typical clinical syndrome despite normal CT scans. Patients were classified as having undefined stroke if they did not fit any of these categories[11]. Functional outcome was measured using the modified Rankin Scale[12]. During hospitalization, neurological evaluation was always done by a single neurologist who applied the study criteria for classification of the patients. All evaluations were performed at the

Department of Neuroradiology.

Laboratory Tests

All blood samples were stored at -80(C and were analyzed simultaneously by technicians who were unaware of the clinical data of the patients.

Brachial-Ankle Pulse Wave Velocity (baPWV)

The baPWV was measured using a volume plethymograph (PWV/ABI ; Colin, Co., Ltd., Komaki, Japan), which simultaneously recorded the PWV, blood pressure, electrocardiogram, and heart sounds[6]. Each subject was examined in the supine position, with the electrocardiographic leads on both wrists, a microphone for detecting heart sounds taped at the left sternal edge, and cuffs on both arms and ankles. The cuffs were connected to a plethysmograph sensor that determined the pulse volume waveform and to an oscillometric pressure sensor that measured the blood pressure. Pulse volume waveforms were recorded using a semiconductor pressure sensor, with the acquisition frequency set at 1,200 Hz. Waveforms for the arm and ankle were stored in 10-sec batches with automatic gain analysis and quality adjustment. The baPWV data were obtained after the subjects had rested for at least 5 min. The reproducibility of baPWV values obtained in healthy subjects was reported to be reasonable, with an interobserver coefficient of variation of 2.4% (n=15) and an intraobserver coefficient of variation of 5.8% (n=17)[6].

Diagnosis of Peripheral Arterial Disease

PAD was diagnosed as follows: Criterion[1] was severe stenosis or occlusion of a lower extremity artery on MRA and/or no diastolic reverse flow (type II - VI) on lower extremity ultrasonography. Criterion 1 was positive when one of these two factors was detected. Criterion[2] was an ankle-brachial index <0.9, a pulseless artery, and/or symptoms of PAD. Criterion 2 was positive when two of these three factors were detected. PAD was defined as present when both criterion 1 and criterion 2 were positive.

Statistical Analysis

Data were recorded on standard forms and then entered into a database. Results are expressed as percentages or as the mean (standard deviation (SD). A nonparametric test (the Mann-Whitney U test) was used to compare variables between groups. Two-way analysis of variance (ANOVA) was used for comparison of the means of numerical variables between three groups. Multiple comparison with the Kruskal-Wallis test was also employed to compare three groups. The risk of ischemic stroke in patients with PAD was estimated by forward stepwise multiple logistic regression analysis with adjustment for the apoprotein A1 level, carotid intima-media thickness (IMT), and modified Rankin scale score (on admission).

Ethics

The design of this study was approved by the Ethics Committee and the Data Protection Committee of Harasanshin General Hospital (Fukuoka, Japan). Informed consent to participation was obtained from all patients (or their closest relatives).

RESULTS

One hundred and thirty-three patients with stroke were evaluated for enrollment in the study, but 32 patients were excluded because of an unclassified stroke subtype (n=13) or refusal to participate (n=19).

Therefore, 101 patients were investigated.

Characteristics of the Subjects

Table 1 shows the characteristics of the 3 subgroups of stroke patients and their risk factors. The mean age of the small artery occlusion group was significantly higher than that of the patients with cardioembolic stroke. The mean serum triglyceride level of the small artery occlusion group was signifi-

Table 1-A Characteristics of the Stroke Patients

Risk Factors	Small artery occlusion (n=73)	Large artery atherosclerosis (n=13)	Cardioembolic stroke (n=15)	P value	Multiple comparison
Age [years, mean±SD]	68.9±12.1[a)]	75.6±9.1	79.3±10.2[b)]	0.0035	a) vs b)**
Male sex [%]	51(69.9%)	9(69.2%)	7(46.7%)	0.2172	
Blood pressure					
Systolic [mean±SD, mmHg]	159.2±26.4	172.5±45.9	167.5±27.9	0.4158	
Diastolic [mean±SD, mmHg]	85.3±14.4	87.5±21.4	87.6±13.8	0.7922	
BMI [kg/m^2]	22.3±2.7	21.8±2.6	22.9±3.3	0.6084	
Smoking [%]	45(61.6%)	11(84.6%)	12(80.0%)	0.1399	
History:					
Hypertension [%]	67(91.8%)	11(84.6%)	14(93.3%)	0.6679	
Diabetes Mellitus [%]	29(39.7%)	4(330.8%)	2(13.3%)	0.1404	
Hyperlipidemia [%]	60(82.2%)	10(76.9%)	12(80.0%)	0.8972	
PAD [%]	57(78.1%)	11(84.6%)	13(86.7%)	0.5869	

ANOVA ; ** $p<0.01$

Table 1-B Characteristics of the Stroke Patients (contined)

Lipids	Small artery occlusion (n=73)	Large artery atherosclerosis (n=13)	Cardioembolic stroke (n=15)	P value	Multiple comparison
TC [mean±SD, mg/dl]	208.3±46.3	212.6±29.7	196.5±40.3	0.5664	
TG [mean±SD, mg/dl]	113.0±86.3[a)]	100.0±64.5	77.0±60.0[b)]	0.0307	a) vs b)*
HDL-C [mean±SD, mg/dl]	45.5±37.5	43.0±36.0	46.0±36.0	0.8837	
LDL-C [mean±SD, mg/dl]	127.1±106.0	137.0±117.8	138.0±92.2	0.6641	
Lipoprotein(a) [mean±SD, mg/dl]	17.1±10.0	23.3±8.83	12.1±7.7	0.4864	
Apoprotein A1 [mean±SD, md/dl]	124.0±112.0	125.5±103.0	119.0±96.0	0.1736	
Apoptotein B [mean±SD, md/dl]	100.0±84.0	103.5±89.0	103.0±79.0	0.5980	
Apoprotein E [mean±SD, md/dl]	4.364±1.097	4.813±1.092	4.100±0.700	0.3149	
RLP-C [mean±SD, md/dl]	3.3±2.7	4.15±3.3	3.6±2.2	0.2736	

TC; total cholesterol TG; Kruskal-Wallis test; * $p<0.05$
HDL-C; HDL cholesterol, LDL-C; LDL cholesterol
RLP-C; RLP cholesterol

Table 1-C Others Factors

Lipids	Small artery occlusion (n=73)	Large artery atherosclerosis (n=13)	Cardioembolic stroke (n=15)	P value	Multiple comparison
CRP [mean±SD, mg/dl]	0.0±0.0[a)]	0.0±0.0[b)]	1.0±0.3[c)]	0.0004	a) vs c)** b) vs c)*
D-D [mean±SD, μg/dl]	0.6±0.3[d)]	1.2±0.4	3.1±0.98[e)]	0.0001	d) vs e)**
TAT [mean±SD, ng/dl]	2.50±1.75[f)]	2.60±1.65	8.65±3.58[g)]	0.0051	f) vs g)**
IMT [mean±SD, mm]	1.110±0.928	1.310±1.015	1.14±0.95	0.2481	
ABI [mean±SD]	1.14±1.02	1.04±0.8	1.15±1.08	0.1281	
baPWV [mean±SD, mmHg]	1934±1650	1847±131	2405±1896	0.1816	
modified Rankin Scale [mean±SD]					
on admission	3.0±2.0	4.0±2.0	5.0±5.0	0.0001	h) vs i)**
on discharge	1.0±0.0	2.0±1.0	4.0±2.0	0.0001	j) vs k)** j) vs l)**
Admission period [mean±SD, days]	23.0±18.0	23.5±20.0	2405±1896	0.0022	m) vs n)**

ANOVA; ** $p<0.01$ Kruskal-Wallis test; * $p<0.05$

CRP; C reactive protein
TAT; thrombin-antithrombin III complex
ABI; ankle brachial index
D-D; D-dimer
IMT; intima-media thickness
baPWV; brachial-ankle pulse wave velocity,

cantly higher than that of the cardioembolic stroke group, although there were no significant differences among the D-dimer (D-D) three stroke subtypes with respect to the other serum lipids. Serum C reactive protein (CRP) and thrombin-antithrombin III complex (TAT) levels were significantly higher in the cardioembolic stroke patients than in those with small artery occlusion, while serum CRP was significantly higher in the cardioembolic stroke patients than in those with large artery atherosclerosis. The modified Rankin scale values on admission and discharge were significantly higher and the duration of admission was significantly longer in the cardioembolic stroke patients than in those with small artery occlusion.

Association Between PAD and Risk Factors

The associations between PAD and various risk factors are displayed in Table 2. PAD was present in 81 of the 101 stroke patients (80.2%), including 57 out of 73 patients with small artery occlusion, 11 out of 13 with large artery occlusion, and 13 out of 15 with cardiogenic embolism. In 42 of these 81 patients (51.9%), PAD was asymptomatic. When the associations between PAD and various risk factors were assessed, no significant differences of these risk factors were found between the stroke patients with and without PAD.

The serum apoprotein A1 level was significantly higher in the stroke patients with PAD than in those without PAD. However,

Table 2-A Association Between PAD and Risk Factors

Risk Factors	PAD		P value
	positive (n=81)	negative (n=20)	
Age [years, mean±SD]	72.0±11.9	68.6±12.6	0.2585
Male sex [%]	52(64.2%)	15(75.0%)	0.5148
Blood pressure			
Systolic [mean±SD, mmHg]	162.8±30.1	159.8±29.5	0.6925
Diastolic [mean±SD, mmHg]	86.7±15.6	82.7±13.3	0.2970
BMI [kg/m²]	22.4±2.8	22.0±2.5	0.5521
Smoking [%]	55(67.9%)	13(65.0%)	1.0000
History:			
Hypertension [%]	75(92.6%)	17(85.0%)	0.5293
Diabetes Mellitus [%]	28(34.6%)	7(35.0%)	1.0000
Hyperlipidemia [%]	68(84.0%)	14(70.0%)	0.2669

Table 2-B Association Between PAD and Risk Factors (continued)

Lipids	PAD		P value
	positive (n=81)	negative (n=20)	
TC [mean±SD, mg/dl]	204.0±176.5	202.0±181.0	0.2585
TG [mean±SD, mg/dl]	107.0±76.3	102.5±79.0	0.6886
HDL-C [mean±SD, mg/dl]	45.0±37.0	55.0±38.0	0.0631
LDL-C [mean±SD, mg/dl]	134.9±106.5	118.8±108.7	0.2833
Lipoprotein(a) [mean±SD, mg/dl]	1.71±10.1	13.1±6.2	0.3644
Apoprotein A1 [mean±SD, md/dl]	121.0±110.0	143.0±116.0	0.0188*
Apoptotein B [mean±SD, md/dl]	100.0±85.5	95.0±81.0	0.4607
Apoprotein E [mean±SD, md/dl]	4.33±1.03	4.52±1.13	0.5266
RLP-C [mean±SD, md/dl]	3.6±2.6	3.7±3.1	0.3438

Mann-Whitney U-test; * $p<0.05$

TC; total cholesterol TG;
HDL-C; HDL cholesterol, LDL-C; LDL cholesterol
RLP-C; RLP cholesterol

Table 2-C Association Between PAD and Risk Factors (continued)

Other Factors	PAD positive (n=81)	PAD negative (n=20)	P value
CRP [mean±SD, mg/dl]	0.2±0.0	0.0±0.0	0.2444
D-D [mean±SD, μg/dl]	0.8±0.4	0.9±0.2	0.4585
TAT [mean±SD, ng/dl]	2.7±1.9	2.4±1.8	0.1809
IMT [mean±SD, mm]	1.15±0.98	0.98±0.83	0.0126*
ABI [mean±SD]	1.13±1.01	1.12±1.08	0.5942
baPWV [mean±SD, mmHg]	1979±1712	1868±1371	0.0608
modified Rankin Scale [mean±SD]			
on admission	3.0±2.0	2.0±1.3	0.0376*
on discharge	1.0±1.0	1.0±0.0	0.1069
Admission period [mean±SD, days]	26.0±19.0	24.0±18.8	0.5650

CRP; C reactive protein
TAT; thrombin-antithrombin III complex
ABI; ankle brachial index
D-D; D-dimer
IMT; intima-media thickness
baPWV; brachial-ankle pulse wave velocity,
Mann-Whitney U-test; * $p<0.05$

there were no significant differences of the other lipid parameters between the patients with and without PAD.

The associations between PAD and several other factors are shown in Table 2 The carotid intima-media thickness and the modified Rankin scale score on admission were significantly larger in the stroke patients with PAD than in those without PAD, but there were no significant differences of the other factors between the patients with and without PAD.

Multiple Logistic Regression Analysis

Logistic regression analysis showed that the apoprotein A1 level and the modified Rankin scale score on admission were strongly related to the occurrence of stroke in patients with PAD (Table 3).

DISCUSSION

The present study showed that PAD is frequently associated with acute ischemic stroke due to either large or small artery occlusion, suggesting that it may be important to perform screening for PAD in patients with ischemic stroke. Our study also revealed that the prevalence of PAD is increased in stroke patients, suggesting that detection of PAD may help to improve the prognosis of patients with ischemic stroke. In general, an ABI of less than 0.9 is considered to indicate the presence of PAD Since blood pressure is higher in the lower limbs than in the upper limbs, the normal ABI ranges from 1.0 to 1.5[13]. Detection of PAD by measuring the ABI was previously found to have a 90% sensitivity and 95% specificity, so this method is generally accepted as the gold standard[14]. In many studies conducted in Europe and the USA, PAD was defined as being present when the ABI was less than 0.9[15)~20)]. Alternatively, an ABI greater than 0.90 at rest that decreases by 20% or more after exercise has been proposed to be diagnostic of PAD[21]. This suggests that patients with leg pain on exertion who have ABI values >0.90 should

Table 3 Forward Stepwise Multiple Logistic Regression Analysis Of PAD in Relation to Apoprotein A 1, IMT, and modified Rankin Scale (on admission)

	Coefficient	Odds ratio	95% CI		P value
Apoprotein A1	−0.02138	0.979	0.959	0.999	0.0312
modified Rankin Scale (on admission)	0.5041	1.660	1.01	2.71	0.0318

be considered for an exercise test. However, it is difficult to do such a test in high-risk patients like the acute ischemic stroke patients in this study, making it difficult to diagnose PAD from the ABI alone, particularly when vessels below the knee are involved. Therefore, we defined PAD as being present when either lower extremity MRA or ultrasonography showed severe stenosis or occlusion, or when at least two out of three clinical factors (ABI <0.9, a pulseless artery, and symptoms) were positive. The prevalence of PAD has varied in previous studies, depending on the age distribution of the subjects and the presence or absence of underlying disease.

The majority of patients with PAD are asymptomatic; in fact, only 22% of them have symptoms like leg pain or intermittent claudication[22]. In the present study, no attempt was made to identify PAD on the basis of symptoms such as intermittent claudication for the following two reasons: 1) it is difficult to distinguish PAD from other diseases based on symptoms alone and 2) PAD is usually asymptomatic (most of our patients had early disease).

Our findings were consistent with the results of some previous studies that have addressed the relationship between PAD and ischemic stroke. Risk factors for an abnormal ABI have been investigated by several authors. In the ARIC study, a high total cholesterol level was found to be a major risk factor for PAD[14]. In another study, the non-HDL cholesterol level was more strongly correlated with ApoproteinB than LDL cholesterol as a predictor of coronary atherosclerosis[23]. We found that both non-HDL and LDL levels were higher in men without PAD, while logistic regression analysis showed that the apoprotein A1 level was strongly correlated with the occurrence of stroke in patients who had PAD. However, this was a small sample size and cross-sectional study, so a causal relationship cannot be deduced from our results. Therefore, a large-scale investigation will be necessary to determine the relationship between PAD and ischemic strok.

PAD not only interferes with daily activities and affects QOL, but also worsens the prognosis of patients with ischemic stroke. The present study revealed that that PAD is frequently associated with ischemic stroke, suggesting that it is important to screen stroke patients for PAD.

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REFERENCES

1) Danesh J, Collins R and Peto R: Chronic infections and coronary heart disease: is there a link? Lancet 350: 430-436, 1997.
2) Lehmann ED: Clinical value of aortic pulse wave velocity measurement. Lancet 354: 528-529, 1999.
3) Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, Target R and Levy BI: Assessment of arterial distensibility by automatic pulse wave velocity measurement: validation and clinical application studies. Hypertension 26: 485-490, 1995.
4) Cohn JN: Vascular wall function as a risk marker for cardiovascular disease. J Hypertens 17 (Suppl 5): S41-S44, 1999.
5) van Popele NM, Grobbee DE, Bots ML, Asmar R, Topouchian J, Reneman RS, Hoeks AP, van der Kuip DA, Hofman A and Witteman JC: Association between arterial stiffness and atherosclerosis: the Rotterdam study. Stroke 32: 454-460, 2001.
6) Yamashina A, Tomiyama H, Takeda K, Tsuda H, Arai T, Hirose K, Koji Y, Hori S and Yamamoto Y: Validity, re-

producibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. Hypertens Res 25 : 359-364, 2002.
7) Newman AB, Siscovick DS and Manorioet AL : Ankle-arm index as a marker of atherosclerosis in the cardiovascular health study : cardiovascular heart study (CHS), collaborative research group. Circulation 88 : 837-845, 1993.
8) Hatano S : Experience from a multicentre stroke register : a preliminary report. Bull World Health Organ 54 : 541-553, 1976.
9) Von Reutern G-M and Budingen HJ : Ultrasound Diagnosis in Cerebrovascular Disease. Stuttgart, Germany/New York, NY : Georg Thieme Verlag ; 1989.
10) De Bray JM and Glatt B : Quantification of atheromatous stenosis in the extracranial internal carotid artery. Cerebrovasc Dis 5 : 414-426, 1995.
11) Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL and Marsh EE 3rd : Classification of subtype of acute ischemic stroke : definitions for use in a multicenter clinical trial : TOAST : Trial of Org 10172 in Acute Stroke Treatment. Stroke 24 : 35-41, 1993.
12) Hacke W, Kaste M, Fieschi C, von Kummer R, Davlos A, Meier D, Larrue V, Bluhmki E, Davis S, Donnan G, Schneider D, Diez-Tejedor E and Trouillas P : Randomized doubleblind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischemic stroke (ECASS II) : Second European-Australasin acute stroke study investigators. Lancet 352 : 1245-1251, 1998.
13) Criqui MH, Denenberg JO, Langer RD and Fronek A : The epidemiology of peripheral artery disease : importance of identifying the population at risk. Vasc Med 2 : 221-226, 1997.
14) Fowkes FG : The measurement of atherosclerotic peripheral arterial disease in epidemiological surveys. Int J Epidemiol 17 : 248-254, 1988.
15) Meijer WT, Hoes AW, Rutgers D, Bots ML, Hofman A and Grobbee DE : Peripheral arterial disease in the elderly : the Rotterdam study. Arterioscler Thromb Vasc Biol 18 : 185-192, 1998.
16) Zheng ZJ, Sharrett AR and Chanbless LE : Associations of ankle-brachial index with clinical coronary heart disease, stroke and preclinical atherosclerosis risk in communities (ARIC) study. Atherosclerosis 13 : 115-125, 1997.
17) Fowkes FG, Housley E, Cawood EH, Macintyre CC, Ruckley CV and Prescott RJ : Edinburgh artery study : prevalence of asymptomatic and symptomatic peripheral artery disease in the general population. Int J Epidemiol 20 : 384-392, 1991.
18) Stoffers HE, Rinkes PE, Kester AD, Kaiser V and Knottnerus JA : The prevalence of asymptomatic and unrecognized peripheral arterial occlusive disease. Int J Epidemiol 25 : 282-290, 1996.
19) Kudoh M, Kimura K, Yamada N and Ishigame M : The frequency and factors promoting arteriosclerosis obliterans in NIDDM patients. Diabetes J 39 : 91-96 (in Japanese), 1996.
20) Chiba S, Yoshida H and Itoh I : Epidemiological survey of arteriosclerosis obliterans by determination of the ankle pressure index. Jpn J Vasc Surg 5 : 549-555 (in Japanese), 1996.
21) Orchard TJ and Strandness DE : Assessment of peripheral vascular disease in diabetes : report and recommendations of an international workshop sponsored by the American Diabetes Association and the American Heart Association September 18-20,1992, New Orleans, Louisiana. Circulation 88 : 819-828, 1993.
22) Ballantyne CM, Andrews TC, Hisa JA, Kramer JH and Shear C : ACSESS study group. Correlation of non-high-density lipoprotein cholesterol with apoprotein B : effect of 5 hydroxymethylglutanyl coenzyme A reductase inhibitors on non-high-density lipoprotein cholesterol levels. Am J Cardiol 88 : 265-269, 2001.
23) Kazunori K, Ueda H, Kasagi F, Masunari N. Epidemiological study on the prevalence of peripheral arterial disease of lower extremities in a fixed population in Hiroshima. The Collection of Kyouei-Seimei Corporation Research Support Papers 13 : 61-65, 1997.

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