Evaluation of the Early Stage of Carotid Atherosclerosis Using the Vascular Response to Nitroglycerin and High-Resolution Ultrasonography

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The purpose of this study was to determine whether the early stages of arterial atherosclerosis could be evaluated by the vascular response to nitroglycerin (NTG). The vascular elasticity of the common carotid artery (CCA) was evaluated at sites without atheromatous plaque using high-resolution ultrasonography before and after sublingual NTG administration. The vascular elasticity was expressed by vascular strain (VS) and pressure-strain elastic modulus (Ep). The study was performed in 9 normal young males (group N) and 52 patients who were suspected to have coronary artery disease (CAD). In group N, VS of the CCA increased from 9 ± 2 to $13 \pm 4\%$, Ep decreased from 80 ± 18 to 42 ± 14 kPa ($p < 0.01$) and systolic blood pressure (SBP) decreased from 116 ± 8 to 109 ± 8 mmHg ($p < 0.05$) after NTG administration. Two different responses were observed in the patients. One group of patients (group A, $n=27$) showed similar responses to NTG as those of group N; that is, VS increased from 8 ± 4 to $14 \pm 8\%$, Ep decreased from 115 ± 64 to 57 ± 31 kPa ($p < 0.01$) and SBP decreased from 121 ± 15 to 110 ± 16 mmHg ($p < 0.05$). In the other group (group B, $n=25$), in spite of SBP decreasing from 124 ± 21 to 111 ± 21 mmHg ($p < 0.05$), VS decreased from 11 ± 4 to $7 \pm 3\%$ and Ep increased from 76 ± 29 to 113 ± 53 kPa ($p < 0.01$). The prevalence and severity of CAD were lower in group A than in group B (33 vs 80%, 11 vs 60%, $p < 0.01$, respectively). The prevalence of atheromatous plaque of the CCA was also lower in group A than in group B (4 vs 22%, $p < 0.01$). These results revealed that the prevalence of carotid atheromatous plaque was highly correlated with that of CAD ($p=0.001$) and suggested that the vascular elasticity response of the CCA to NTG is highly associated with atherosclerotic changes of the artery and may be a useful method for detecting the early stages of atherosclerosis. (Jpn Circ J 1998; 62: 494–498)

Key Words: Atherosclerosis; Nitroglycerin; Vascular elasticity

Atherosclerosis is a series of histopathological changes that includes intimal thickening, degenerative compositional changes of the medial layer (sclerotic change) and atheroma-formation (atherotic change).¹ The recent development of high-resolution B-mode ultrasonography has made it possible to detect and evaluate the atheromatous changes of the arteries, particularly the carotid artery.^{2–5} However, despite these studies, methods that evaluate the sclerotic change of an artery, an early stage of atherosclerosis, have not been established. Vascular sclerotic changes increase vascular stiffness, and may disturb the vascular responses to a vascular relaxant. Although decreased elasticity of the arterial wall in the atherosclerotic carotid artery has been reported,^{6–9} the carotid artery response to vascular relaxant has not been reported yet.

Nitroglycerin (NTG), a relaxant of vascular smooth muscle, may change arterial elasticity.^{10,11} If the change in elasticity of the atherosclerotic artery in response to NTG is different from that of the normal artery, evaluation of this

response may be valuable in assessing atherosclerotic changes. The purpose of the present study was to determine whether the early atherosclerotic changes of the common carotid artery (CCA) could be evaluated by the response of vascular elasticity to NTG, where there was no ultrasonic atheromatous plaque. The vascular elasticity was expressed by vascular strain (VS) and pressure-strain elastic modulus (Ep). Moreover, the extent of atherosclerosis of the CCA has been shown to be strongly correlated with the extent of coronary artery disease (CAD).^{12–16} Therefore, we also examined whether the decreased elasticity response of the CCA to NTG was associated with CAD.

Methods

Study Subjects

The study was performed in 52 patients who were admitted for the evaluation of CAD (35 men and 17 women, aged from 45 to 74 years old), and 9 normal male volunteers (aged from 23 to 27 years old) without risk factors for atherosclerosis such as hyperlipidemia, hypertension, glucose intolerance and smoking. Patients with frequent arrhythmias, congestive heart failure, congenital and acquired heart disease were excluded, because the hemodynamic abnormality of these diseases may affect the diameter change of the CCA. Patients with recent cerebrovascular attacks and a blood pressure difference between the bilateral brachial artery of greater than 10 mmHg were also excluded. Informed consent was obtained from every

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Fig 1. The cursor of the M-mode was placed 3 cm proximal to the carotid bifurcation where there was no atheromatous plaque (upper), and the maximum diameter of the artery were recorded before (CONTROL) and after (NG) nitroglycerin sublingual administration. Dd, end diastolic diameter; Ds, end systolic diameter.

patient.

All the 52 patients were suspected to have CAD and underwent coronary arteriography to assess the extent of it. A significant stenosis was defined as a greater than 50% decrease in the internal diameter. There were 29 patients with significant coronary stenosis.

Examination of the Carotid Arteries

None of the patients stopped their regular medications, such as nitrates, calcium antagonists and/or beta blockers, for fear of ischemic and withdrawal hypertensive attacks. The protocol began in the morning, 2 h after medications were taken.

The ultrasonographic studies were performed using a B-mode and M-mode ultrasonographic instrument (SSD-7255, Aloka, Tokyo, Japan) with a 10 MHz frequency of the mechanical sector scanner. Subjects were placed in the supine position with their head tilted slightly to the right, and the left CCA was investigated. We scanned the CCA from the carotid bifurcation to the proximal portion as far as possible in 3 longitudinal sections (anterior oblique, lateral and posterior oblique) and in transverse section, and the presence of atheromatous plaque was evaluated. Atheromatous plaque was thought to be present if the

Table 1 Hemodynamic and Vascular Elasticity Responses to NTG in Normal Young Males

No.	Age (years)		SBP/DBP (mmHg)	PP (mmHg)	D (mm)	VS (%)	Ep (kPa)
1.	25	C	122/58	64	6.8	9	97
		NTG	116/79	37	6.7	12	42
2.	25	C	108/70	38	6.6	8	68
		NTG	101/64	37	6.3	8	64
3.	27	C	118/78	40	5.3	9	60
		NTG	110/78	32	5.3	14	74
4.	23	C	132/71	61	6.1	10	84
		NTG	125/81	44	6.3	11	55
5.	26	C	114/69	45	6.6	6	103
		NTG	106/73	33	6.6	8	56
6.	26	C	110/58	52	5.4	7	101
		NTG	104/67	37	6.2	14	36
7.	24	C	115/66	49	6.2	13	52
		NTG	105/72	33	6.0	18	25
8.	25	C	108/56	52	5.4	9	79
		NTG	104/67	37	5.9	18	29
9.	24	C	118/63	55	6.6	10	75
		NTG	112/72	40	6.6	15	37

C, before nitroglycerin; NTG, after NTG administration; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; D, internal diameter of common carotid artery; VS, vascular strain; Ep, pressure-strain elastic modulus.

echogenic lesion was detected in at least one of the longitudinal sections and in the transverse section. In order to measure the diameter of the CCA, the cursor of the M-mode was placed approximately 3 cm proximal to the carotid bifurcation, where there was no ultrasonic evidence of atheromatous plaque, and the maximum diameter of the artery was recorded. The pulse rate (PR) and blood pressure (BP) of the brachial artery were noninvasively recorded every minute using a monitoring system (STBP-680 Nippon Colin Inc., Tokyo, Japan).

Administration of Nitroglycerin

NTG was given sublingually (0.3 mg) and BP were recorded every minute thereafter. When the maximal BP response was obtained, the diameter of the CCA was recorded.

Data Analysis

The internal diameter (D) of the CCA was measured in 3 consecutive cardiac cycles of M-mode echograms. Optimal gain setting of the sonograph was obtained to measure the internal diameter from the leading edge of the intimal echo of the near arterial wall to the leading edge of the intimal echo of the far wall. The smallest and the largest internal diameters were measured at end-diastole and peak systole, respectively, as shown in Fig 1. Two independent observers measured twice the internal diameter of all ultrasonograms of the CCA, with a 1-week interval. The interobserver reliability expressed as the correlation between these 2 observers was satisfactory ($r=0.85$). Paired t-test was used to compare the intraobserver differences in the measurement of carotid D. The results showed that the value measured by these 2 observers was constant ($p>0.05$). Intraobserver differences for the measurement were compared using paired t-test. There was no significant difference between these 2 observers ($p>0.05$). The VS and Ep of the CCA were calculated according to the following formulae:

Table 2 Hemodynamics, Arterial Diameter, Vascular Elasticity of Group N and Patient Groups A and B Before and After NTG

Group		SBP/DBP (mmHg)	PP (mmHg)	PR (bpm)	D (mm)	VS (%)	Ep (kPa)
N	C	116±8 /65±7	51±9	68±11	6.1±0.6	9±2	80±18
	NTG	109±8*/73±6	37±4 [†]	70±12	6.2±0.4	13±4 [†]	42±14 [†]
A	C	121±15 /69±11	52±12	69±12	6.2±1.0	8±4	115±64
	NTG	110±16*/70±11	40±12 [†]	71±11	6.1±0.8	14±8 [†]	57±31 [†]
B	C	124±21 /66±14	58±15	67±11	6.1±1.3	11±4	76±29
	NTG	111±21*/68±10	43±11 [†]	69±12	6.2±1.4	7±3 [†]	113±53 [†]

Mean ± SD;

C, before nitroglycerin; NTG, after nitroglycerin administration; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; PR, pulse rate; D, end diastolic internal diameter of common carotid artery; VS, vascular strain; Ep, pressure-strain elastic modulus; **p*<0.05 vs data before NTG in the same group; [†]*p*<0.01 vs data before NTG in the same group.

Table 3 The Prevalence of Atheromatous Plaque of the Common Carotid Artery and the Extent of Coronary Artery Disease in Groups A and B

	Group A (n=27)	Group B (n=25)
Averaged age (years)	57±10	62±10*
Atheromatous plaque (persons, %)	6 (22%)	21(84%)*
Coronary artery disease (persons, %)	9 (33%)	20(80%)*
single vessel disease	8 (30%)	8(32%)
multi vessel disease	1 (4%)	12(48%)*

**p*<0.01 vs group A.

Table 4 The Prevalence Atheromatous Plaque and the Extent of Coronary Artery Disease in Group A and B After Age Matched Comparison

	Group A (n=14)	Group B (n=22)
Atheromatous plaque (persons, %)	4 (29%)	17 (77%)*
Coronary artery disease (persons, %)	3 (21%)	17 (77%)*
single vessel disease	3 (21%)	5 (23%)
multi vessel disease	0 (0%)	12 (55%)*

**p*<0.01 vs group A.

VS (%)=(systolic D - diastolic D) mm/diastolic D mm¹⁷
Ep (kPa)=(systolic BP - diastolic BP) mmHg/7.6 mmHg per VS⁷

The data were expressed as mean ± SD (standard deviation). Paired t-test was used to compare hemodynamics, end diastolic D, VS and Ep before and after NTG administration. Chi square test was used to analyze the prevalence of CAD and carotid atheromatous plaque between groups. A probability of less than 0.05 was considered significant.

Results

Table 1 reveals the clinical data of the 9 normal young males (group N) before and after NTG administration. BP, pulse pressure (PP) and Ep decreased, while VS increased after NTG in all subjects but one, in whom only strain was unchanged (case No 2). Hemodynamics, diastolic D, VS and Ep were compared in group N and the 52 patients with suspected CAD before and after NTG administration (Table 2). In group N, the SBP, PP and Ep decreased significantly (*p*<0.05, *p*<0.01, *p*<0.01, respectively), and VS increased significantly (*p*<0.01) after NTG. However, no significant change in PR and diastolic D were found after NTG administration.

In the patients who underwent coronary arteriography for the evaluation of CAD (n=52), BP and PP decreased after NTG administration, as in group N. However, VS and Ep changed in various ways. We divided the patients into 2 groups by the different responses to NTG: (i) there were 27 patients (18 men and 9 women; mean age, 57±10 years) who showed a decrease in Ep and an increase in VS, as in group N (group A), and (ii) 25 patients (17 men and 8 women; mean age: 62±10 years) who showed an increase in Ep and a decrease in VS (group B). There were also no significant changes in diastolic D before and after NTG administration among the patients in these groups. As in

group N, NTG decreased SBP and PP significantly in both group A and B. The changes in SBP and PP were not significantly different when compared between any 2 of the 3 groups. In group A, Ep decreased from 115±64 to 57±31 kPa (*p*<0.01) and VS increased from 8±4 to 14±8% (*p*<0.01). In group B, Ep increased from 76±29 to 113±53 kPa (*p*<0.01), and VS decreased from 11±4 to 7±3% significantly (*p*<0.01). However, there were no statistical differences in the basal values of Ep and VS when compared between any 2 of the 3 groups.

The extent of atheromatous plaque in the carotid artery and CAD were compared between groups A and B (Table 3). Atheromatous plaque of the carotid artery was not observed in any group N subject. The prevalence of atheromatous plaque was substantially lower in group A than in group B (22 vs 84%, *p*<0.01). In group A, there were 9 patients (33%) with CAD and only 1 patient had multivessel disease (4%). In group B, however, there were 20 patients (80%) with CAD and 12 patients (48%) had multivessel disease. The prevalence and severity of CAD were statistically significant when compared between group A and B (*p*<0.01). Because the average age of patients in group B was higher than in group A, an age-matched comparison was made and is shown in Table 4. Even after correction for age, the prevalence of carotid atheromatous plaque and CAD were significantly higher in group B than in group A (77 vs 29%; 77 vs 21%, respectively, *p*<0.01).

The prevalence of carotid atheromatous plaque was highly correlated with CAD (*p*=0.001).

Discussion

The present study has shown that in patients suspected of having CAD there were 2 different responses of the CCA to NTG, when the vascular elasticity was evaluated at a site without ultrasonic evidence of atheromatous plaque. One

group of patients (group A) showed a decrease in Ep and an increase in VS after NTG administration, similar to the response in group N, whereas the other group (group B) showed an increase in Ep and a decrease in VS, although SBP and PP decreased after NTG administration in both groups. It appeared that group B had more extensive and severe atherosclerotic changes of the carotid and coronary arteries than group A. These results indicate that the Ep and VS responses of the CCA to NTG were strongly associated with atherosclerotic changes.

The reliability of this study was examined. High resolution ultrasonography with a 10MHZ mechanical scanner was used to evaluate the internal diameter change of the carotid artery and this system provides a longitudinal resolution of 0.2mm. Moreover, the measured value of 2 independent observers was consistent ($p>0.05$). However, instead of intracarotid pressure, the brachial artery was used to measure the BP. The limitation of the present study was that the relationship between the brachial artery BP and intracarotid pressure was not evaluated, because of the risk of directly measuring intracarotid pressure.

The extent of atherosclerosis of the CCA has been evaluated by morphological changes, including plaque formation and intima-media thickness (IMT).⁴ Atherosclerotic changes may alter the physiological properties of the artery wall even where morphological changes are minimal.⁸ Moreover, Barth et al have evaluated the pulsatile change of the carotid artery diameter and shown that the pressure-strain modulus was greater at the atherosclerotic lesion than at the nonatherosclerotic sites.⁸ Thus, it is possible that the physiological properties of the artery were modified by vasoactive drugs, with which early atherosclerotic changes could be clarified, but this has not been reported previously. We evaluated the changes in VS and Ep of the CCA by the effects of NTG, and have shown that even at the nonatheromatous sites the responses were significantly different between patients with and without extensive atherosclerosis.

VS is a percent change of vascular diameter during the systolic phase, which is determined by BP, especially systemic BP (SBP), and vascular wall properties.¹⁸⁻²⁰ Vascular wall is composed of smooth muscle and connective tissue components.^{18,19} In the elastic type of artery, vascular elasticity is mainly modulated by the smooth muscle component.^{18,21} We also measured the Ep to estimate the arterial elasticity, because Ep is determined with data that normalize for PP and vascular diameter differences between subjects, and is commonly used in population surveys.⁷ A previous experiment showed that NTG decreases the pulse-wave velocity of the aorta, suggestive of a decrease in arterial stiffness.¹⁰ As the vascular elasticity is determined by BP and vascular properties,¹⁸⁻²⁰ the decreased pulse-wave velocity might be due to a NTG-induced decrease in BP, rather than by vascular smooth muscle relaxation. However, 0.3mg of NTG was administered sublingually in our study and, although we did not examine the plasma concentration of NTG during the study, the decrease in SBP and PP was similar between groups A and B. Therefore, the different responses of VS and Ep to NTG between these 2 groups can not be explained by a decrease in BP induced by NTG.

In the present study, although NTG decreased SBP, VS increased and Ep decreased in groups N and A. These results suggest that NTG decreases the elasticity of CCA in those patients who have less atherosclerotic changes

through the direct effect of NTG on vascular smooth muscle, which results in an increase in vascular elasticity. In group B, VS decreased and Ep increased after NTG administration. Although the reason for this is unclear, it indicates that the relaxing effect of NTG may have been limited in spite of the fact that atheromatous plaque was not detected at the measured sites of the CCA. It has been reported that the IMT measurement was a useful method for detecting the early stage of atherosclerosis.^{4,22} Increased IMT may be an important factor in disturbing the effect of NTG, although IMT was not evaluated in the present study.

Finally, our results identified that there was a high correlation between CAD and atheromatous plaque,^{13,16} and revealed that it is possible to evaluate the vascular atherosclerotic change at an early stage using this noninvasive and safe method. Moreover, this method does not use expensive instruments. From point of view of preventing the progression of atherosclerosis, it is an easy and useful method for evaluating early stage vascular atherosclerosis.

Vascular atherosclerosis regresses after hyperlipidemia is adjusted.^{22,23} Further studies are needed to determine whether the vascular elasticity response to NTG will change if the risk factors for atherosclerosis are controlled.

References

1. Blankenhorn DH, Kramsch DM: Reversal of atherosclerosis and sclerosis. The two components of atherosclerosis. *Circulation* 1989; **79**: 1-7
2. Crouse JR, Harpold GH, Kahl FR, Toole JF, McKinney WM: Evaluation of a scoring system for extracranial carotid atherosclerosis extent with B-mode ultrasound. *Stroke* 1986; **17**: 270-275
3. Salonen R, Salonen JT: Progression of carotid atherosclerosis and its determinants: a population-based ultrasonography study. *Atherosclerosis* 1990; **81**: 33-40
4. Salonen JT, Salonen R: Ultrasound B-mode imaging in observational studies of atherosclerotic progression. *Circulation* 1993; **87**(Suppl II): 56-65
5. Zanette E, Bozzao L, Buttinelli C, Mariottini A, Pappata S, Lenzi GL: High resolution real-time B-mode echography in the diagnosis of extracranial carotid lesions: Comparison with traditional angiography. *Acta Neurochir* 1987; **84**: 43-47
6. Spagnoli LG, Bonanno E, Mauriello A, Zanette E, Faraglia V, Palmieri G, Fiorani P: Histopathological characterization of carotid plaque echotomography. *Int Angiol* 1988; **7**: 75-80
7. Riley WA, Freedman DS, Higgs NA, Barnes RW, Zinkgraf SA, Berenson GS: Decreased arterial elasticity associated with cardiovascular disease risk factors in the young: Bogalusa Heart Study. *Arteriosclerosis* 1986; **6**: 378-386
8. Barth JD, Blankenhorn DH, Wickham E, Lai JY, Chin HP, Selzer RH: Quantitative ultrasound pulsation study in human carotid artery disease. *Arteriosclerosis* 1988; **8**: 778-781
9. Hirai T, Sasayama S, Kawasaki T, Yagi S: Stiffness of systemic arteries in patients with myocardial infarction. *Circulation* 1989; **80**: 78-86
10. Latson TW, Hunter WC, Katoh N, Sagawa K: Effect of nitroglycerin on aortic impedance, diameter, and pulse-wave velocity. *Circulation Res* 1988; **62**: 884-890
11. Smulyan H, Mookherjee S, Warner RA: The effect of nitroglycerin on forearm arterial distensibility. *Circulation* 1986; **73**: 1264-1269
12. Crouse JR, Thompson CJ: An evaluation of methods for imaging and quantifying coronary and carotid lumen stenosis and atherosclerosis. *Circulation* 1993; **87**(Suppl II): 17-33
13. Craven TE, Ryu IE, Espeland MA, Kahl FR, McKinney WM, Toole JF et al: Evaluation of the associations between carotid artery atherosclerosis and coronary artery stenosis: A case-control study. *Circulation* 1990; **82**: 1230-1242
14. Pasquale GD, Andreoli A, Pinelli G, Grazi P, Manini G, Tognetti F et al: Cerebral ischemia and asymptomatic coronary artery disease: A prospective study of 83 patients. *Stroke* 1986; **17**: 1098-1101
15. Salonen JT, Salonen R: Ultrasonographically assessed carotid morphology and the risk of coronary heart disease. *Arterioscler Thromb* 1991; **11**: 1245-1249
16. Wofford JL, Kahl FR: Howard GR, McKinney WM, Tool JF, Crouse JR 3rd. Relation of extent of extracranial carotid artery atherosclerosis

- sis as measured by B-mode ultrasound to the extent of coronary atherosclerosis. *Arterioscler Thromb* 1991; **11**: 1786–1794
17. Aberg AK, Axelsson J: Some mechanical aspects of an intestinal smooth muscle. *Acta Physiol Scand* 1965; **64**: 15–27
 18. Dobrin PB, Rovick AA: Influence of vascular smooth muscle on contractile mechanics and elasticity of arteries. *Am J Physiol* 1969; **217**: 1644–1651
 19. Hayashi K, Sato M, Handa H, Moritake K: Biomechanical study of the constitutive laws of vascular walls. *Exp Mechanics* 1974; **14**: 440–444
 20. Cox RT: Comparison of arterial wall mechanics in normotensive and spontaneously hypertensive rats. *Am J Physiol* 1979; H159–H167
 21. Cox RT: Alterations in active and passive mechanics of rat carotid artery with experimental hypertension. *Am J Physiol* 1979; H597–H605
 22. Byington RP, Furberg CD, Crouse JR, Bond MG: Pravastatin, lipids, and atherosclerosis in the carotid arteries (PLACII). *Am J Cardiol* 1995; **76**: 54C–59C
 23. Farrar DJ, Green HD, Wagner WD, Bond MG: Reduction in pulse wave velocity and improvement of aortic distensibility accompanying regression of atherosclerosis in the Rhesus monkey. *Circ Res* 1980; **45**: 425–432