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Histological Evaluation of Coronary Plaque in Patients With Variant Angina: Relationship Between Vasospasm and Neointimal Hyperplasia in Primary Coronary Lesions

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Objectives. **This study was designed to determine whether coronary vasospasm in patients with variant angina pectoris (VAP) may produce focal organic lesions at the site of vasospasm that would contribute to disease progression.**

Background. **Recent clinical angiographic and experimental studies have demonstrated the potential role of vasospasm in the worsening of organic coronary stenosis.**

Methods. **We studied histologically the coronary plaques obtained at atherectomy in 202 patients with moderate to severe coronary stenosis. This population included 22 patients with VAP, 100 patients with chronic stable angina and 80 patients with restenosis following angioplasty or atherectomy. Diagnosis of VAP was based on both the clinical feature of angina at rest associated with ST elevation and a positive response to acetylcholine provocation test.**

Results. **The most common histological appearance in 92% of patients with stable angina was hypocellular fibroatheromatous plaques, whereas neointimal hyperplasia was the characteristic feature of the plaque observed in 90% of patients with restenosis. The coronary specimens at the site of spasm in 15 of the 22 patients (68%) with VAP demonstrated intimal injuries such as neointimal hyperplasia (15), thrombus formation (2), and intimal hemorrhage (3). Neointimal hyperplasia was significantly more common in the patients with VAP as compared with those with stable angina (68% vs. 8%; $p < 0.0001$). A rapid progression of organic stenosis within three years was angiographically found in 5 of the 22 patients with variant angina. In all five cases, neointimal hyperplasia was the main contributor to the worsening of the organic lesion at the site of spasm. These histological findings in patients with VAP extremely resembled those in restenosis. Except for vasospasm, no factors significantly predicted the presence of neointimal formations in primary coronary lesions.**

Conclusions. **Coronary vasospasm may provoke vascular injury that leads to the formation of neointima in VAP patients similar to that seen with restenosis. Coronary spasm may thus play a key role in the rapid coronary stenosis progression in certain patients with VAP.**

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Thrombosis complicated by the disruption of plaque is considered to be the most important mechanism for the development of acute coronary syndrome (1–3). The disruption of plaque is thought to expose the platelet aggregates present on a surface erosion or ulcerated atheromatous plaque leading to the release of vasoconstrictor substance (3–5). Although the cause of the disruption of plaque remains to be elucidated, coronary vasospasm as well as shear stress can compress the atherosclerotic plaques to disrupt them and cause vascular injuries (6,7). It is well known that acute myocardial infarction or sudden death may occur in patients with variant angina, even with no or insignificant organic coronary stenosis. Recent clinical studies (8,9) have shown that patients with variant angina treated with calcium blockers rarely experience cardiac events; the presence of severe organic stenosis greatly increases the risk of unstable angina and acute myocardial infarction. Several angiographic studies concerning the relationship between vasospasticity and the acceleration of atherosclerosis have yielded conflicting results (10–14). Coronary angioscopic studies in patients with variant angina have revealed a high frequency of such intimal injuries as intimal flap, hemorrhage, and ulcer at the sites of vasospasm that were not detected by the simultaneous performance of coronary arteriography (15). The "response-to-injury hypothesis" holds that vascular injury may be important in the pathogenesis of progressive atherosclerosis by causing the release of several growth factors (16). While several autopsy studies have been done, few studies have evaluated histological findings in patients with variant angina (17–19). We therefore evaluated histologic findings in coronary atherosclerotic lesions at the site of vasospasm in patients

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Abbreviations and Acronyms
ECG = electrocardiogram, electrocardiographic
VAP = variant angina pectoris

with variant angina to determine whether coronary spasm may lead to vascular injury.

Methods

Patients. We obtained 356 specimens of coronary arteries for histological analysis from 350 consecutive patients with symptomatic coronary artery disease. These patients had undergone directional coronary atherectomy between January 1993 and September 1996 at The Cardiovascular Institute and Hospitals of Juntendo University. In 6 of the 350 patients, coronary lesions of two vessels were treated simultaneously by atherectomy. Patients with unstable angina, myocardial infarction, or insufficient coronary atherectomy specimens were excluded from this study. A total of 202 patients was enrolled in this study, 187 males and 19 females, aged 29 to 83 years, mean 60.0 ± 9.3 years. All patients had evidence of significant narrowing (more than 75% luminal stenosis) of major coronary arteries, which were responsible for angina pectoris. Specimens were obtained from 22 consecutive patients (including one autopsied case) with variant angina, 100 patients with chronic stable angina, and 80 patients with interventional restenosis (postangioplasty 46 patients, postatherectomy 34 patients).

Definition. Variant angina was defined as angina pectoris that occurred at rest, usually at night and/or the early morning, associated with a transient elevation of ST segment greater than 2 mm on the electrocardiogram (ECG) either during a spontaneous angina attacks or during a provocation test. Focal occlusive or near-occlusive coronary artery spasm at the site of organic stenotic lesion was documented angiographically during a spontaneous angina attacks or during the acetylcholine spasm provocation test (20,21). The change in the ST segment during this test exhibited an extent and distribution similar to that observed during the anginal attack and/or the exercise tolerance test before coronary angiography with the provocation test. The intracoronary administration of isosorbide dinitrate quickly reversed the coronary arterial spasm and electrocardiographic changes and relieved the chest pain in all patients.

Chronic stable angina pectoris was defined as angina on exertion associated with ST segment depression >1 mm during an exercise tolerance test without previous infarction. Interventional restenosis was defined as chronic restenosis within one year after the patients had undergone angioplasty or atherectomy.

Coronary angiography with spasm provocation test. Continuous electrocardiographic monitoring was done following admission. Patients stopped calcium antagonists and oral nitrates for at least 48 h before the performance of coronary angiography. The latter was performed by the Judkins technique with monitoring of the ECG and blood pressure in the early morning while patients were in the fasting state. Coronary angiograms of the left and right coronary arteries were obtained in the right and left anterior oblique projections after performing simultaneous biplane left ventricular cineangiography. After we had obtained the control coronary angiograms, we injected 3 incremental doses of acetylcholine chloride (20, 50, and 100 μg) dissolved in 5 ml of warm 0.9% saline. The drug was injected directly into the left coronary artery through the Judkins catheter in patients with spontaneous angina that had often occurred at rest (20–22). The left coronary angiographic examination was performed immediately upon the development of chest pain and/or a change in ST segment. In the absence of chest pain or ST change, the examination was done at 3 and again at 6 min after each injection. Acetylcholine was injected into the right coronary artery in 2 incremental doses of 20 and 50 μg, and the right coronary angiogram was similarly obtained. The resulting coronary spasm was immediately relieved by infusing 1 to 2 mg isosorbide dinitrate into the involved coronary artery. Another coronary angiogram was then obtained. Results of the acetylcholine test to provoke coronary spasm were considered to be positive only in the presence of a total or subtotal obstruction of the coronary artery with a greater than 2 mm depression, or elevation, of ST segments. The distribution and extent of ST segment changes on ECG during the spasm provocation test resembles those during attacks of variant angina and/or exercise tolerance tests (23) before angiography. Therefore, we considered the resulting coronary spasm during the provocation test to be responsible for variant angina attacks before the performance of angiography. The percent stenosis of the diameter of the coronary artery was measured with calipers and graded according to the American Heart Association classification (24). Full informed consent was obtained from each patient for the cardiac catheterization, the provocation test for the induction of coronary spasm and, as necessary, therapeutic atherectomy. This procedure was performed with the Simpson AtheroCath (Devices for Vascular Intervention Inc., Redwood City, California) one to two months after the diagnostic coronary angiogram was obtained. Atherectomy was performed at the site of organic stenosis at which spontaneous or provoked coronary spasm had been confirmed angiographically. In one patient with typical variant angina who died suddenly just before the atherectomy, a postmortem coronary angiogram was obtained by selectively injecting the right ostium with a mixture of barium and gelatin. This was followed by radiography after fixing the tissue in 10% neutral-buffered formalin for 15 min at a pressure of 100 mmHg. The major epicardial coronary arteries were then cut transversely at 3- to 5-mm intervals. The proximal segments of the right coronary arteries that showed >50% cross-sectional luminal stenosis on visual inspection were considered responsible for the variant angina. Such tissues were examined in detail histologically.

Preparation of atherectomy samples. Specimens obtained at atherectomy (or autopsy) were immersed in Carnoy solution for 24 h, dehydrated in a graded series of alcohol and xylene, and embedded in paraffin blocks. Histological sections were stained with hematoxylin and eosin, azan-Mallory, Movat's pentachrome, Mallory's phosphotungstic acid hematoxylin, and alcian blue (pH 2.5) stains.

Immunohistochemical staining was performed with mouse monoclonal antibodies to smooth muscle actin and to KP-1 (specifically targeted against the cytoplasm of the mouse macrophage). Both antibodies were obtained from Dako Laboratories, Copenhagen, Denmark. They were used at a dilution of 1:100 using the standard techniques (avidin-biotin-peroxidase, ABC method). All sections were evaluated independently by three pathologists (H.S., S.K., and S.S.), who were blind to the patient's clinical history and type of angina pectoris.

Definitions of the components of the resected plaque. The depth of the resected coronary specimens was identified by light microscopy using the sections stained with Movat's pentachrome. We then investigated the incidence of the following in the coronary atherectomy specimens, as described by other authors (25,26): fibroatheroma (necrotic debris), mural thrombus, neointimal hyperplasia, intimal hemorrhage, calcification, and subintimal tissue. Necrotic debris was identified as an extracellular area rich in lipids that consisted mainly of amorphous material with an abundance of cholesterol clefts surrounded by clusters of macrophages and lipid-laden foam cells. Thrombus was identified as aggregates of platelets, strands of fibrin, and erythrocytes with sites of mural attachment. Intimal hemorrhage was recognized as aggregates of erythrocytes with deposits of hemosiderin. In the sections stained with hematoxylin and eosin, calcified deposits were detected as focal, red-purple, amorphously stained areas of the thickened intima. Neointimal hyperplasia was identified by the proliferation of stellate-shaped smooth muscle cells within a myxedematous stroma with extracellular matrix.

Statistical analysis. All data were verified by a retrospective review of the patients' records. Data are expressed as proportion or means and SD for continuous variables. Univariate analysis was done on all clinical variables to detect any possible correlation between the patient's clinical findings and the characteristics of tissue obtained at atherecthomy (or autopsy). Statistical comparisons utilized the chi-square statistic, or, when appropriate, Fisher's exact test, for categorical variables, and Student's *t* test for continuous variables. A level of $p < 0.05$ was considered statistically significant.

Results

Clinical characteristics of patients with variant angina. The baseline clinical characteristics and angiographic findings of the patients with variant angina are shown in Table 1. Demographic data for the patients with variant angina and those with chronic stable angina pectoris appear in Table 2. Two groups did not differ significantly as to age, sex, target lesion, coronary risk factors, and weight of tissue obtained at atherectomy (except for the autopsied case, patient No. 10).

Coronary spasm was documented at the site of organic stenosis with a 75–90% reduction in lumen diameter by angiography in the 22 patients with variant angina (Fig. 1). Coronary spasm was provoked by the intracoronary injection of acetylcholine in 20 of the 22 patients with variant angina, and occurred spontaneously in the other 2 patients (Nos. 1 and 5). Four of the twenty-two patients with variant angina (Nos. 1, 2, 11, and 16) showed a rapid progression of coronary stenosis within 3 years at the previously spastic segment, as observed on follow-up angiograms; except for smoking, these four patients lacked coronary risk factors. Autopsy examination of a fifth patient (No. 10), who had died suddenly of unstable angina, also showed evidence of the rapid progression of coronary stenosis, as compared with the coronary arteriograms obtained 2 months earlier (Fig. 1 and 2). This 63-year-old man (patient No. 10) had suffered recurrent attacks of chest pain at rest with reversible ST elevations in II, III, aVF and complete AV block for 2 months. The attacks responded rapidly to the intravenous administration of isosorbide dinitrate and nifedipine. Coronary angiography revealed single-vessel coronary disease with 75% stenosis of the luminal diameter of the right coronary artery in its proximal portion and a positive response to the acetylcholine provocation test that showed a decrease in the lumen diameter from 75% to 99% with ST elevation. Although the patients received appropriate medications, he developed a severe attack of chest pain while traveling that was unresponsive to nitroglycerin. He died suddenly two months later. Five patients showed a rapid progression of organic coronary stenosis at the site of spasm (Fig. 3). Of the 22 patients, 19 had one-vessel disease, and 3 patients (Nos. 3, 6, and 16) had two-vessels disease (Table 1). One of those three (No. 6) had a total occlusive lesion and two (Nos. 3 and 16) had a nonspastic, 50% stenotic lesion. These coronary lesions were not analyzed histologically.

Histological findings of coronary plaque in variant angina. The most common histopathologic findings of the coronary plaque in the patients with chronic stable angina were hypocellular fibroatheromatous tissue with necrotic debris, observed in the lesions of 92 (92%) of the 100 patients. Neointimal hyperplasia was the most characteristic histological finding in the plaque in 78 (90%) of the 80 patients with chronic restenosis after intervention (Table 3).

However, 15 (68%) of the 22 coronary atherectomy (or autopsy) specimens, resected from patients with variant angina, showed evidence of intimal injury such as neointimal hyperplasia with infiltration by inflammatory cells, mainly lymphocytes and macrophages. Two of the fifteen specimens had mural thrombus and three had intimal hemorrhage in the fibrocellular thickening of the intima (Fig. 2 and 4).

In contrast, only 8% of the 100 coronary specimens from the patients with chronic stable angina showed such neointimal formation, a highly significant difference between groups ($p < 0.0001$). Such neointimal hyperplasia was observed in all coronary specimens obtained from the four patients (Nos. 1, 2,

Table 1. Clinical Characteristics and Angiographic Results of the Patients With Variant Angina

Case No.	Age (yr)	Sex	FU (mo)	HP (mo)	Trend	ST ↑ (leads)	Angiographic findings				Spasm-provocation test	Distribution and extent of ST change during the provocation test	Coronary risk factors	Follow-up angiography before atherectomy
							Coronary disease	Organic lesion site	Stenosis (control)	Stenosis during spasm				Stenosis progression (interval)
1	44	M	12	2	crescendo	V2-6	1 VD	LAD #7	90%	100%	spontaneous	V3-6	smoking	50% to 90% (12 mo)
2	66	M	3	3	crescendo	V1-4	1 VD	LAD #6	90%	99%	positive	V1-4	smoking	25% to 90% (6 mo)
3	66	M	3	2	crescendo	V1-4	2 VD	LAD #6	90%	99%	positive	V1-4	smoking, HL	N/A
4	53	M	6	2	crescendo	V3-5	1 VD	LAD #6	75%	99%	positive	V1-4	smoking	N/A
5	56	M	3	1	crescendo	V2-5	1 VD	LAD #7	90%	99%	spontaneous	V2-5	0	N/A
6	58	M	18	1	crescendo	V3-5	2 VD	LAD #7	90%	99%	positive	V1-4	smoking, DM	N/A
7	61	M	1	1	crescendo	V1-4	1 VD	LAD #7	75%	99%	positive	V2-4	smoking	N/A
8	68	M	5	2	crescendo	V1-4	1 VD	LAD #6	90%	99%	positive	V2-4	HT, DM	N/A
9	42	M	8	3	crescendo	Holter (V5)	1 VD	LAD #7	75%	100%	positive	V1-4	smoking	N/A
10	63	M	2	2	crescendo	II, III, aVF	1 VD	RCA #2	75%	99%	positive	II, III, aVF	0	50% to 75% (2 mo)
11	66	M	7	4	crescendo	V1-4	1 VD	LAD #6	75%	100%	positive	V1-4	smoking	0% to 75% (7 mo)
12	64	M	4	4	crescendo	II, III, aVF	1 VD	RCA #3	75%	100%	positive	II, III, aVF	0	N/A
13	66	M	10	2	crescendo	V1-4	1 VD	LAD #6	75%	100%	positive	V1-4	smoking	N/A
14	68	F	12	4	crescendo	Holter (V5)	1 VD	LAD #6	90%	99%	positive	V2-4	HT	N/A
15	58	M	48	1	crescendo	V1-4	1 VD	LAD #6	75%	100%	positive	V1-4	0	50% to 75% (3 yr)
16	59	M	15	3	crescendo	V4-5	2 VD	LAD #7	75%	100%	positive	V3-5	0	N/A
17	61	M	3	none	stabilized	V5-6	1 VD	LAD #9	90%	100%	positive	V1-5	smoking, HT	N/A
18	53	M	4	none	stabilized	I, aVL	1 VD	LAD #9	90%	100%	positive	II, III, aVF	smoking	N/A
19	67	M	5	none	stabilized	II, III, aVF	1 VD	RCA #3	90%	99%	positive	II, III, aVF	smoking	0% to 90% (9 yr)
20	45	M	3	none	stabilized	V3-5	1 VD	LAD #6	90%	100%	positive	V1-4	smoking	N/A
21	49	M	10	none	stabilized	V2-6	1 VD	LAD #6	75%	100%	positive	V1-4	0	N/A
22	73	M	24	none	stabilized	V1-4	1 VD	LAD #6	75%	100%	positive	V1-4	HT	N/A

HP = Hot phase, which indicates a period of extremely high disease activity (more than one attack per week) (13,14). FU = duration of follow-up until coronary atherectomy. VD = indicates coronary vessel disease; LAD = left anterior descending coronary artery; RCA = right coronary artery; HL = hyperlipidemia; DM = diabetic mellitus; HT = hypertension. Trend indicates whether angina attacks had worsened (cresendo) or stabilized on therapy. mo = months; yr = year.

11, and 15) who demonstrated a rapid progression of coronary stenosis on angiography. The postmortem examination of patient No. 10, who died suddenly and underwent autopsy, also revealed neointimal proliferation and an occlusive mural thrombus formation in the coronary plaque at the site of spasm in the right coronary artery. The thickened intima consisted of two layers: an inner layer of myxedematous neointimal hyperplasia ("new" plaque) and an outer layer of organized fibromuscular connective tissue ("old" plaque) (Fig. 2-upper). These histological findings were consistent with those of the atherectomy specimens from patients with variant angina. However, case No. 19 with stabilized variant angina, who had shown on angiography a progression from 0 to 90% fixed coronary stenosis over a 9-year period, exhibited common atherosclerotic lesions without neointimal formation.

No significant relation was observed between the occurrence of neointimal hyperplasia and persistent vasospastic activity before the atherectomy. Intimal injury was found in 12 of the 16 patients with crescendo variant angina (average 2.8 attacks per week) as compared with such findings in three of the six patients with variant angina (average 0.7 attacks per week), who had been medically stabilized by medical treatment for several months before undergoing atherectomy, not a significant difference ($p = 0.07$).

Discussion

Previous histological study of coronary plaque obtained by atherectomy. While neointimal hyperplasia is a common finding in restenotic coronary atherectomy specimens obtained postangioplasty, and is generally recognized as a vascular remodeling response to vascular injury, previous atherectomy studies (27–29) have noted that neointimal hyperplasia may also be found in primary coronary lesions. However, such studies did not identify any clinical or angiographic differences between the patients having primary coronary stenoses with or without neointimal hyperplasia. Flugelman et al. (29) identified neointimal hyperplasia with the expression of fibroblast

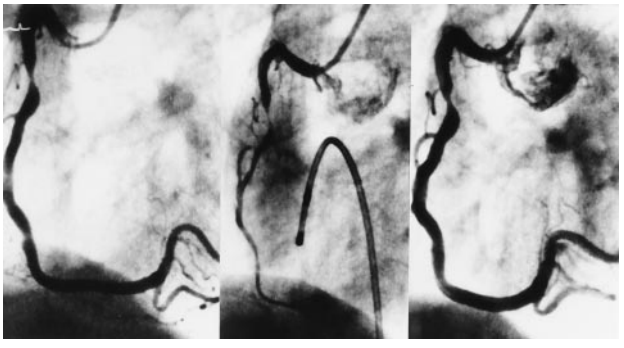
Table 2. Baseline Characteristics of the Patients With Primary Coronary Lesions

	VAP (n = 22)	SAP (n = 100)	p value
Age, years	60.1 ± 8.3	60.4 ± 9.6	0.52
Male, n(%)	21 (96%)	86 (86%)	0.22
Target vessel of CAD, n(%)			
LAD	19 (86%)	68 (68%)	0.09
RCA	3 (13%)	22 (22%)	0.38
LCx	0 (0%)	10 (10%)	0.12
Tissue weight, mg	17 ± 9*	18 ± 11	0.51
Coronary risk factors			
Hyperlipidemia, n(%)	3 (14%)	46 (46%)	0.05
Diabetic mellitus, n(%)	3 (14%)	18 (18%)	0.62
Hypertension, n(%)	4 (18%)	38 (38%)	0.08
Smoking, n(%)	13 (59%)	66 (66%)	0.54

*Average tissue weight of 21 atherectomy specimens, excluded the autopsied case. Plus/minus values are mean ± SD. VAP = variant angina pectoris; SAP = chronic stable angina pectoris. Values in parenthesis are percents. LAD = left anterior descending coronary artery; RCA = right coronary artery; LCx = left circumflex coronary artery; CAD = coronary artery disease. Hyperlipidemia was defined if TC, TG, LDL, HDL, and Lp(a) values > 220 mg/dl, >200 mg/dl, >130 mg/dl, <35 mg/dl and >30 mg/dl, respectively, after >12 hr of fasting, or if patients were receiving antihyperlipidemic drug treatment or both. TC = total cholesterol; TG = triglyceride; LDL = low density lipoprotein cholesterol; HDL= high density lipoprotein cholesterol; Lp(a) = lipoprotein (a). Hypertension was defined as systolic and diastolic pressure values >140 mm Hg and/or >90 mm Hg, respectively, on three occasions; the patient was receiving antihypertensive treatment or both.

growth factors in plaques that were resected from 18 (56%) of 32 patients with unstable angina but was not detected in any of the 10 patients with stable angina. Those authors suggested that neointimal hyperplasia, related with several growth factors, may play a key role in the transformation from stable to unstable angina. They also suggested that the formation of neointima may lead to a gradual expansion of plaque and thereby, to luminal narrowing and unstable angina, as an alternative mechanism to that of plaque rupture and thrombus formation in a subset of patients with unstable angina. However, the mechanisms that trigger the neointimal hyperplasia in the patients whose angina changed from stable to unstable angina were not identified. To our knowledge, no previous studies have investigated the presence or absence of vasospasticity in coronary lesions targeted for atherectomy. The finding of neointimal hyperplasia in the primary lesion in the present study was significantly more common in the patients with variant angina (15 of 22 patients [68%]) than in the patients

Figure 1. Cineangiograms of focal coronary spasm occurring at the site of significant atherosclerosis (Patient No. 10). **Left,** coronary angiogram of the right coronary artery in left anterior oblique view shows severe coronary stenosis with a 75% reduction of lumen diameter in the midportion. **Middle,** results of the acetylcholine provocation test were positive with the lumen diameter decreasing from a 75% to a subtotal occlusion by the superimposed focal coronary spasm. **Right,** luminal dilatation of the right coronary artery with a residual fixed coronary lesion of approximately 50–75% stenosis was observed after the administration of intracoronary isosorbide dinitrate. Unfortunately, this patient died suddenly of his disease. Autopsy studies were conducted.



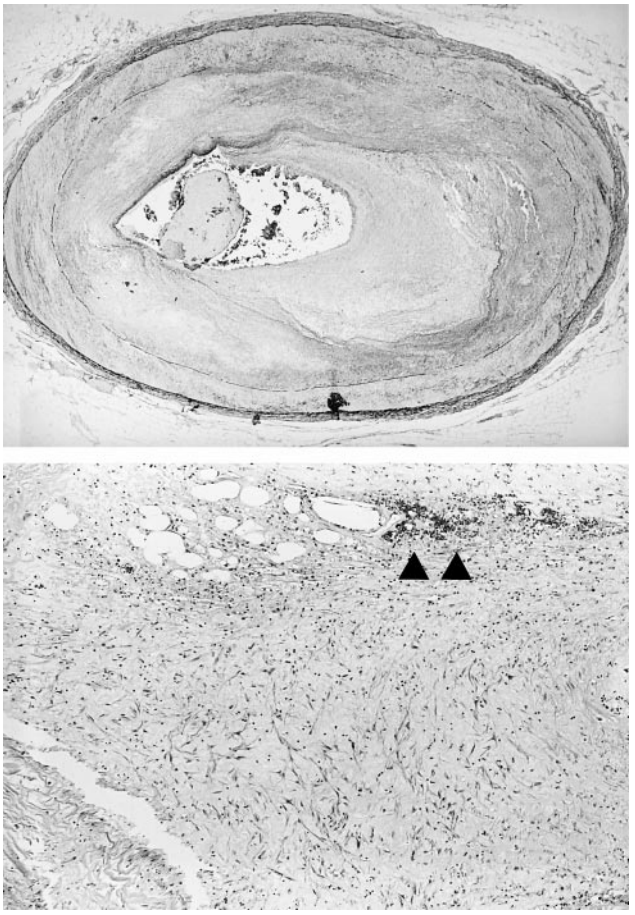


Figure 2. Photomicrographs obtained at autopsy of the coronary arterial site responsible for variant angina in patient No. 10. Histological examination of the affected segment of the right coronary artery shows a focal obstructive fibrous plaque with a superimposed thrombus. There are two distinct intimal layers in the plaque. The inner, light-colored layer, is a myxedematous area of the neointima with an abundance of stellate smooth muscle cells and a considerable amount of extracellular matrix. The outer layer, dark-colored layer, consists of so-called "old" plaque that is mainly composed of relatively hypocellular dense fibrous connective tissue **(upper).** Neointimal hyperplasia with inflammatory cell infiltration is observed in the inner layer, and resembles the histological features in the atherectomy specimens obtained from 14 other patients with variant angina in the present study. Intimal hemorrhage **(arrow)** is also observed **(lower).** (**Upper;** elastica von Gieson stain, ×4. **Lower;** hematoxylin and eosin stain, ×60.)

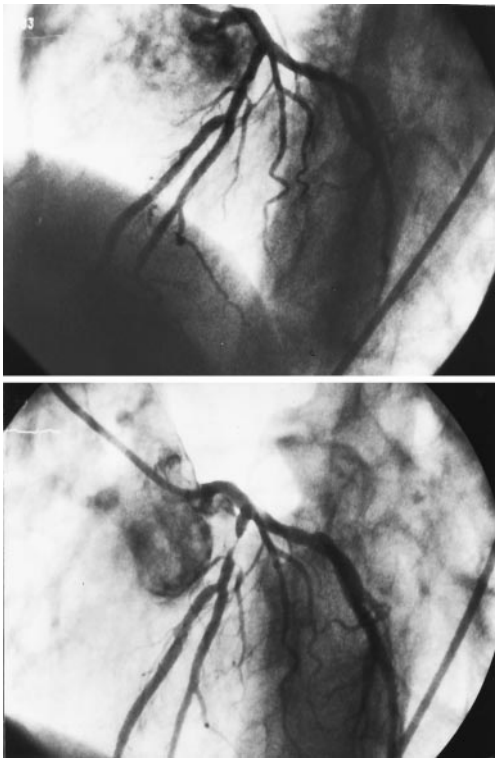


Figure 3. Progressive narrowing of the lumen of the coronary artery in patient No. 2 after 6 months. Coronary angiogram of the left coronary artery (in left cranial view) shows mild coronary stenosis with a 25% reduction in lumen diameter in the proximal portion after the intracoronary administration of isosorbid. Occlusive vasospasm was found at the same site at that time **(upper).** Six months later, rapid progression from a 25% to a 90% fixed coronary stenosis was observed at the previously spastic segment after the intracoronary administration of isosorbide in the follow-up angiogram **(lower)**.

with chronic angina pectoris (8 of 100 patients [8%]). Other than vasospasm, no obvious clinical factors appeared to be predictive of the presence of neointima formation in the primary coronary lesion. We also observed that neointimal hyperplasia was an important contributor to the rapid progression of organic coronary stenosis at site of spasm within three years, as observed on sequential angiograms in four cases of atherectomy as well as in one autopsied case, who had exhibited persistent (crescendo) variant angina for several months before the histologic examination.

Table 3. Histological Findings

	VAP (n = 22)	SAP (n = 100)	Restenosis (n = 80)
Fibro-atheromatous plaque (n)	32% (7)*	92% (92)	10% (8)*
Neointimal hyperplasia (n)	68% (15)*	8% (8)	90% (78)*
Thrombus (n)	10% (2)	6% (6)	9% (11)
Intimal hemorrhage (n)	14% (3)	4% (4)	8% (6)
Calcification (n)	10% (2)	11% (11)	11% (9)

*$p < 0.0001$, versus SAP. VAP = variant angina pectoris; SAP = chronic stable angina pectoris; Restenosis = post-interventional restenosis. Values in parenthesis are total counts.

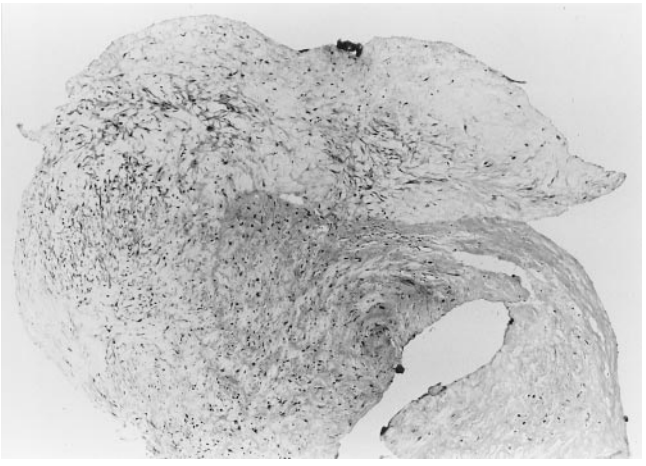


Figure 4. Photomicrograph of atherectomy specimens obtained from the patient No. 2. The proliferation of characteristic spindle-shaped smooth muscle cells (neointimal hyperplasia) is observed in a fibrous plaque (hematoxylin and eosin, ×40).

Similar neointimal hyperplasia has been observed at autopsy in victims of variant angina and sudden death (17–19). Although, based on their observations, Corrado et al. (19) suggested a cause-and-effect relationship between accelerated atherosclerosis and enhanced coronary vasoreactivity, such observations involved only isolated cases. The relationship between vasospasticity and acceleration of atherosclerosis should be further investigated by comparing histologic findings in a large number of patients with organic coronary lesion, both with and without variant angina.

Experimental and clinical studies. An experimental study by Gertz et al. (30) showed that, following the partial ligation of the common carotid arteries in animal models, endothelial damage complicated by platelet aggregation and thrombus formation occurred at the site of focal arterial vasoconstriction. Those authors suggested that recurrent vasospasm may result in endothelial damage and thrombus formation, as well as arterial occlusion, at the site of spasm. Recent studies by Nagasawa et al. (31) and Kuga et al. (32), working with a model of coronary arterial spasm in the miniature pig, provide further experimental evidence that coronary spasm could induce intimal hemorrhage at the affected site. Those authors observed that repeated strong spasm produced hemorrhage and thickening of the intima that frequently resulted in acute myocardial infarction in their animal model. They concluded that intimal hemorrhage, derived from the capillaries in the plaque compressed by spasm, increases the intraplaque pressure and the volume of the plaque, resulting in a progression of the organic coronary stenosis.

In a clinical study using angioscopy, Mizuno et al. (15) also observed vascular injury including intimal flap, ulceration, dissection, and hemorrhage of the intima at the site of vasospasm in 4 of 10 patients with vasospastic angina that was not detected by simultaneous angiography. They suspected that six other patients with variant angina who did not exhibit macroscopic vascular injury might have had microscopic injury.

Our present histological investigation demonstrated such injury to the intima as neointimal hyperplasia, mural thrombus formation, and hemorrhage at the site of spasm in 15 of the 22 patients with variant angina. Our microscopic study showed a higher frequency of vascular injury at site of spasm than observed in their macroscopic study as they suggested in the literature.

Mural thrombus formation and intimal hemorrhage are commonly observed immediately after vascular injury. In contrast, neointimal hyperplasia is a chronic and common form of vascular remodeling after injury, whether the injury is mechanical, as seen following various coronary arterial interventions (33–35), chemical, as seen with exposure to toxic substances (36), or immunologic, as seen in the coronary arteriopathy observed in some patients following heart transplantation (37), as well as in some cases of vasculitis (38). The similar histological picture observed in patients with variant angina and in those with restenosis in the present study indicates that similar pathophysiological mechanisms are operant in both of those groups. Neointimal formation in the plaque at the site of spasm may follow the organization of thrombi, intimal hemorrhage, as well as the rupture of plaque triggered by vasospasm in patients with variant angina, especially during the so-called hot phase (13,14).

Study limitations. Several limitations of this study should be considered. First, only a small number of patients with variant angina were evaluated. Also, we did not investigate cases of organic stenosis with a less than 75% reduction of lumen diameter, even if its organic lesion was located at the site of spasm. Second, we did not carry out a longitudinal study to investigate angiographically the presence or absence of the progression of organic stenosis at the site of recurrent spasm in all cases of variant angina. Third, we did not perform the spasm provocation test in patients with chronic stable angina. Our study did not compare the histological findings in coronary lesions at the sites with spasm vs. sites without spasm. Finally, we could not fully evaluate the composition and structure of the underlying plaque, in that atherectomy specimens do not allow one to explore the entire plaque. Also, the sample may become fragmented during the performance of atherectomy.

Conclusion. The present study suggests a possible role for coronary vasospasm in provoking vascular injury. Our findings suggest that the neointimal hyperplasia associated with coronary vasospasm may be the underlying mechanism for the rapid progression of coronary artery disease in certain patients with variant angina. Prospective studies of a large number of patients with variant angina are required to define clearly the relation between coronary vasospasm and vascular injury leading to progressive atherosclerosis.

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