Impaired Flow-Mediated Vasodilation of Epicardial Coronary Artery in Vasospastic Angina

To evaluate whether the flow-mediated vasodilation and coronary flow reserve are impaired or not in patients with vasospastic angina (VA), we measured the changes of epicardial coronary artery diameter and flow reserve in spasm related-left anterior descending coronary artery (LAD). The flow mediated-response of epicardial coronary arteries in 15 VA were compared with 15 controls. Using quantitative coronary angiography, we measured the diameter of proximal (pLAD) and middle segment (mid-LAD) of LAD under baseline conditions, during increased blood flow after distal adenosine injection and after proximal administration of nitroglycerin. An increased fraction of average peak velocity after injection of adenosine was similar in both groups [control 340 (mean) $\pm$ 24 (SEM)%; VA 330 $\pm$ 19%]. Flow-mediated vasodilation was preserved in all controls (pLAD 13.1 $\pm$ 1.4%; mid-LAD 15.8 $\pm$ 2.5%) but it was significantly impaired in patients with VA (pLAD $-1.0 \pm 1.8\%$; mid-LAD 0.1 $\pm$ 3.5%). The vasodilator response to nitroglycerin was comparable in controls (pLAD 25.8 $\pm$ 2.8%; mid-LAD 27.2 $\pm$ 2.8%) and VA (pLAD 26.2 $\pm$ 5.2%; mid-LAD 26.7 $\pm$ 3.5%). Coronary flow reserve is preserved in patients with VA. However, the flow-mediated response of spasm related-epicardial coronary artery is impaired. This may play an important role in the pathogenesis of coronary artery spasm.

Key Words: Vasodilation, flow mediated; Coronary vasospasm; Vasospastic angina

Seung-Ho Kang, Hoon-Ki Park,
Cheol Whan Lee, Jae-Joong Kim,
Myeong-Ki Hong, Seong-Wook Park,
Seung-Jung Park

Department of Medicine, Asan Medical Center,
University of Ulsan, Seoul, Korea

Address for correspondence
Seung-Jung Park, M.D., Ph.D.
Department of Medicine, Asan Medical Center,
University of Ulsan, 388-1, Pungnap-dong,
Songpa-gu, Seoul 138-736, Korea
Tel: +82.2-224-3150, Fax: +82.2-486-5918

INTRODUCTION

Vasospastic angina has been demonstrated to be caused by epicardial coronary artery spasm (1). Endothelial dysfunction has been regarded as a cause of coronary artery spasm in patients with vasospastic angina since they have impairment of vasodilation in response to various vasoactive amines (2-7). However, it is still controversial whether coronary artery spasm results from defective endothelium-dependent vasodilation or local hyperreactivity of vascular smooth muscle (8, 9). Previous studies have focused on autonomic nervous system abnormality (2) or local vascular hyperreactivity to various stimuli (12-13). In most in vivo studies to date, acetylcholine has been a major tool for evaluating endothelial function. However, acetylcholine may induce the direct vasoconstriction of vascular smooth muscle irrespective of whether endothelial dysfunction is present or not.

Flow-mediated vasodilation is an important physiologic mechanism in the regulation of coronary blood flow. This phenomenon could provide flow-mediated dilator feedback to oppose the constrictor force of the myogenic response to increased intraluminal pressure, thereby coordinating vasomotor tone changes in the coronary network (14). The flow-mediated vascular response is thought to be more physiologic than those to acetylcholine in the evaluation of vasomotor function. This response was shown to be an endothelium-dependent phenomenon (14-17). Several studies have demonstrated the adequacy of this technique as a method to evaluate endothelial-dependent vasomotor function (18-22). However, there is little attention about flow-mediated vasodilation in patients with vasospastic angina. Thus, the present study was designed to evaluate the flow-mediated vascular response and coronary flow reserve in spasm related-epicardial coronary artery of patients with vasospastic angina.

MATERIALS AND METHOD

Patients

Fifteen patients with vasospastic angina and fifteen

patients with atypical chest pain were studied. Patients with unstable angina, recent myocardial infarction, left ventricular dysfunction, and risk factors with coronary artery disease were excluded. Patients, with chest pain suggesting vasospastic angina and normal 201-thallium labeled myocardial perfusion scans, were enrolled in vasospastic angina group. All patients underwent bedside 'intravenous ergonovine echocardiography' after discontinuation of all cardioactive drugs for at least 3 days.

Previously we reported intravenous ergonovine echocardiography as an accurate noninvasive method for diagnosis of coronary artery spasm (23, 24). A spasm site predicted by intravenous ergonovine echocardiography was well correlated with angiographically confirmed spasm site. Especially, a spasm site of LAD (left anterior descending coronary artery) territory by intravenous ergonovine echocardiography was well coincided with those confirmed by coronary angiogram [concordance rate, 25/27 (93%)] (24). Patients with regional wall motion abnormalities of mid-LAD (middle segment of LAD) territory on intravenous ergonovine echocardiogram were selected as vasospastic angina group for this study.

All control patients with atypical chest pain had negative intravenous ergonovine echocardiography and angiographically normal coronary arteries. Written informed consents were obtained from all patients before the study.

Diagnostic coronary angiography was performed in all patients before the study by using 7F catheters with a standard femoral artery approach. Three thousand units of heparin was given intravenously after arterial puncture. Non-ionic contrast material was used for all patients. Patients were excluded from the study if they had spontaneous coronary artery spasm during coronary angiogram, or angiographically proved atherosclerotic coronary artery lesion. Thirty-five patients of vasospastic angina were initially included for this study. Of these patients, 20 patients were excluded from the study (7 patients showed spontaneous coronary artery spasm during the study; 13 patients had atherosclerotic coronary artery lesion). Since spontaneous coronary artery spasm occurs more frequently during the early morning hours (25-28), all studies were performed in the late morning or afternoon (11 AM to 1 PM) to reduce the likelihood of confounding spontaneous spasm.

Study protocol

After the diagnostic angiogram, an additional 5,000 units of heparin was given intravenously, and an 8F guiding catheter was positioned in the ostium of the left coronary artery. A 3F infusion catheter was advanced through the guiding catheter into the proximal portion of distal LAD. A 0.014-inch Doppler flow wire (15 MHz)

was then advanced through the infusion catheter and the tip of the Doppler flow wire was positioned distal to the end of the infusion catheter. Before beginning the study protocol, the position of the Doppler flow wire was adjusted to obtain an optimal and stable flow velocity signal. The Doppler wire and infusion catheter position were not changed thereafter. Throughout the study, heart rate, aortic pressure, mean and phasic flow velocity were monitored continuously. A projection for optimal visualization of the left coronary artery was chosen and unchanged throughout the study. After the control condition had been established, adenosine was injected as a bolus into the distal LAD through the infusion catheter. The dose of adenosine (1.5 cc, 18 μg) was chosen on the basis that maximal flow is achieved by a bolus of 18 μg adenosine (29). Coronary angiography was performed with an injection of 8-10 mL of nonionic contrast medium at control conditions and 60 seconds after the peak flow velocity induced by adenosine (21, 30, 31). We waited 5 minutes after each angiogram to exclude the effect of contrast-induced vasodilation. After return to baseline conditions, 250 μg nitroglycerin was injected into the left main coronary artery through the guiding catheter. Last coronary angiogram was performed 4 minutes after nitroglycerin injection.

Quantitative coronary angiography

Coronary angiograms were recorded on a 35-mm cinefilm (30 frames/s) using a Siemens cineangiographic system on the projections that allowed the best visuals of the LAD on end-diastolic frames without overlap of side branches. Angiograms were obtained at quiet end expiration to avoid possible effects of respiration. Flow-mediated vasodilation was evaluated in the mid-portion of the proximal LAD (pLAD) and mid-LAD free of side branches. The proximal segment of circumflex artery was used as control. A portion at least 5 mm proximal to the infusion catheter tip was selected for quantitative analysis of mid-LAD segment. Measurement of the lumen diameter of each arterial segment was performed quantitatively with the aid of a cinevideodensitometric analysis system (automatic edge-detection method). The arterial segments under study were videodigitized at end-diastolic and then stored in the cardiac image analysis system. The diameter of the segment of interest was measured with twofold magnification, and the average value from triplicate measurements was used for analysis. Two or more fixed anatomical structures serving as references were determined to allow assessment of serial changes in the diameter of the same arterial site. Each angiogram was analyzed at random by experienced investigators unaware of the clinical history. The percentage changes in the coronary dia-

meter was calculated as follow: (diameter after drug - control diameter)/ control diameter $\times 100$ (%).

Statistical analysis

All data were expressed as mean $\pm$ SEM. Statistical comparisons between the two groups of patients were made by the unpaired Student t test. Serial changes of hemodynamic variables and coronary vessel diameters at baseline, after adenosine and after nitroglycerin injection were evaluated by ANOVA for repeated measures. A p value less than 0.05 was considered statistically significant.

RESULTS

Clinical characteristics

Table 1 summarizes the clinical characteristics of the patients. The baseline characteristics, such as age, sex, total cholesterol levels, HDL cholesterol levels, were comparable between the two groups.

Hemodynamic variables

Resting heart rate, and arterial blood pressure were also similar in both groups (Table 1). There was no significant change in heart rate, and arterial blood pressure during the injections of adenosine or nitroglycerin (data not shown).

Flow-mediated vasodilation

All subjects included in this study had angiographically normal coronary artery. Intracoronary injection of adenosine into the distal LAD produced significant increase in

average peak flow velocity in both control and vasospastic angina groups (control $340 \pm 24\%$; vasospastic angina $330 \pm 19\%$). And the ratio of resting-to-maximal blood velocity (coronary flow reserve) was similar in both groups. Mean baseline diameter of pLAD and mid-LAD segments were similar in both groups (Table 1). In control subjects, the luminal diameter of both segments significantly increased after intracoronary injection of adenosine (pLAD $13.1 \pm 1.4\%$; mid-LAD $15.8 \pm 2.5\%$). In contrast, flow-mediated diameter changes of both segments were significantly blunted in patients of vasospastic angina (pLAD $-1.0 \pm 1.8\%$; mid-LAD $0.1 \pm 3.5\%$, $P < 0.05$) (Fig. 1). There was no dilation of the left circumflex coronary artery segments in any subjects of both groups after intracoronaty injection of adenosine. In response to nitroglycerin infusion, proximal and mid-LAD segments were dilated to a similar degree in both controls (pLAD $25.8 \pm 2.8\%$; mid-LAD $27.2 \pm 2.8\%$) and vasospastic angina groups (pLAD $26.2 \pm 5.2\%$; mid-LAD $26.7 \pm 3.5\%$).

DISCUSSION

The major finding of this study is that flow-mediated vasodilation is impaired in spasm-related human epicardial left anterior descending coronary artery, whereas the capability of the artery to dilate in response to nitroglycerin is preserved. This result suggests a specific functional alteration in the vasoregulatory capacity of the endothelium in the arteries responsible for vasospastic angina. It has been demonstrated that an increase of blood flow through normal coronary arteries results in vasodilation in animal and human studies. This response has been reported to be dependent on the presence of an intact endothelium and mediated by enhanced release of endothelium-derived relaxing factor (14-17). Under

Table 1. Clinical characteristics of the study population

Characteristic	Vasospastic angina (n=15)	Control (n=15)	P value
Age (yr)	51.6 ± 3.4	47.4 ± 2.5	NS
Sex (male/female), number	10/5	9/6	NS
Total cholesterol (mmol/L)	4.13 ± 0.23	4.29 ± 0.30	NS
Total triglyceride (mmol/L)	1.42 ± 0.11	1.40 ± 0.17	NS
HDL-C (mmol/L)	1.10 ± 0.18	1.14 ± 0.15	NS
SBP (mmHg)	129 ± 6.4	131 ± 5.7	NS
DBP (mmHg)	73 ± 5.1	76 ± 4.4	NS
Heart rate (beats/min)	72 ± 2.9	73 ± 3.4	NS
Baseline APV (mm/sec)	169.0 ± 19.4	162.0 ± 15.4	NS
Vessel size of pLAD (mm)	3.4 ± 0.2	3.5 ± 0.2	NS
Vessel size of mLAD (mm)	2.8 ± 0.1	2.9 ± 0.2	NS

APV indicates average peak velocity; mid-LAD, middle segment of left anterior descending coronary artery; NS, nonsignificant; pLAD, proximal segment of left anterior descending coronary artery.
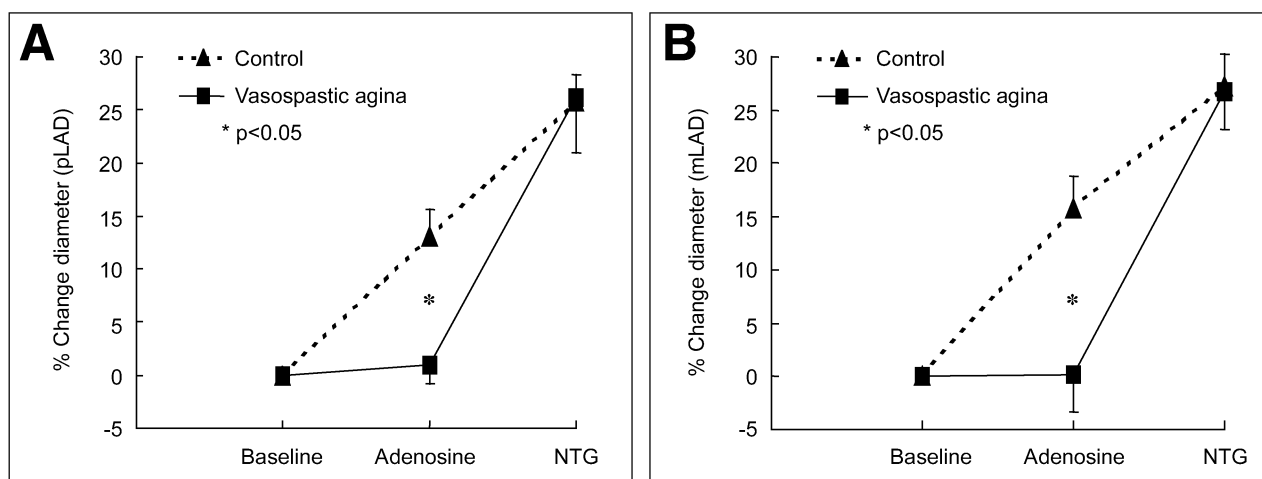
Fig. 1. Comparison of flow-mediated vasodilation and nitroglycerin-induced vasodilation in proximal (A) and middle segments (B) of left anterior descending coronary artery (LAD). Flow-mediated vasodilation was significantly impaired in vasospastic angina patients as compared with the control subjects, whereas nitroglycerin-induced vasodilation was similar in both groups (pLAD, indicates proximal segment of LAD; mid-LAD, middle segment of LAD; NTG, nitroglycerin).

normal conditions, flow-mediated vasodilation represents the major mechanism by which the epicardial vessels respond to stimuli, such as exercise that increases myocardial work and oxygen demand. An important functional consequence of impaired flow-mediated vasodilation of epicardial coronary artery is that it limits blood flow to the myocardium, which may contribute to the development of myocardial ischemia. The incidence of myocardial ischemia in vasospastic angina markedly increases in the early morning (25-28). Although the mechanisms responsible for this increased incidence are not fully understood, we think that the blunted flow-mediated dilating capacity of the epicardial coronary artery probably contributes to aggravation of coronary artery spasm. This hypothesis is supported by the fact that the endothelium plays an important role in modifying fluctuations in coronary vasomotor tone in response to various physiological stimuli that occur in a circadian fashion (32). In this study, we could not perform the study in the early morning (7-9 AM) because spontaneous coronary artery spasm developed in almost all cases. This observations is compatible to previous report on diurnal variation in vasospastic angina (25-28). Unfortunately, in patients with vasospastic angina, the precise mechanism of coronary artery spasm remains unclear. Endothelium-dependent vasodilation mediated by acetylcholine has been reported to be abnormal at the vasospastic site (2-4). However, coronary vasodilation evoked with bradykinin or substance P was comparable to the coronary spasm site induced by ergonovine maleate and the control site. The results suggest that endothelial function is normal at the site of the acetylcholine-induced coronary artery spasm in patients with vasospastic angina (8, 9). In

the animal model of coronary artery spasm, vascular smooth muscles from the spastic segments also show markedly augmented response to histamine and serotonin as compared to those of the nonspastic segments (10, 11). Therefore, it is still unclear whether abnormal vasomotor response results from endothelial dysfunction or local hyperreactivity of vascular smooth muscle in epicardial coronary artery. Acetylcholine has been a major tool for investigating endothelium-dependent vasodilator response but it has some limitations because acetylcholine has a direct vasoconstriction effect on vascular smooth muscle and it is not a physiologically circulating substance. In contrast, flow-mediated vasodilation is both a sensitive and physiological response. Therefore, the present study extended our knowledge of endothelial function in vasospastic angina by showing that flow-mediated vasodilation is impaired in spasm related-epicardial coronary artery. These abnormalities represent endothelial dysfunction rather than a more general impairment of epicardial dilator capacity because these vessels respond normally to nitroglycerin. Coronary artery spasm was reported to occur more frequently in areas of atherosclerotic narrowing (33). Therefore, it has been suggested that in these patients the basic abnormality may be endothelial dysfunction associated with atherosclerosis process. However, the cause cannot be attributed solely to atherosclerosis since the majority of patients with vasospastic angina have normal or near normal coronary arteries in Korea (34) and acetycholine do not provoke spasm in most patients with fixed coronary artery disease (35). The endothelial dysfunction of the spasm related-epicardial coronary artery seems to be diffusely involved. The dilatory response to nitroglycerin of the artery with spasm

in patients with vasospastic angina did not differ from that of either the artery without spasm in the same patients or the coronary artery in the control subjects (36). In this study, flow-mediated response was impaired in both spasm (mid-LAD) and nonspasm site (pLAD) of spasm related-epicardial coronary artery. In this regard, our study supports the previous observations that endothelium dependent-vasodilation of spasm related-epicardial coronary artery is diffusely impaired in patients with vasospastic angina (35, 36).

Coronary flow reserve can be calculated as the ratio of resting-to-maximal blood flow velocity, in the situation which the cross-sectional area of the vessel at the site of Doppler probe placement remains constant between measurements. Despite several limitations, including superimposed mild obstruction of the vessel by the catheter, this method is widely used (37). In this study, an increased fraction of average peak velocity (coronary flow reserve) was similar in both groups. This findings demonstrates that, despite impaired flow-mediated vasodilation of epicardial conductance vessel, flow reserve of the coronary microvasculature is normal in patients with vasospastic angina.

In this study, spasm-related epicardial coronary artery was not documented angiographically because of ethical considerations. However, we previously reported that ergonovine echocardiography can be reliably used to predict the vasospasm segment in VA (23, 24). Finally, we did not evaluate the flow-mediated vasodilation of non-spasm related epicardial coronary artery in VA. Therefore, we do not know whether the flow-mediated vasodilation of nonspasm related-epicardial coronary artery is impaired or not.

In conclusion, the vasomotor tone of intact coronary arteries appears to be modulated by changes in blood flow. Flow reserve of the coronary microvasculature is preserved in patients with vasospastic angina. However, the flow-mediated vasodilation of spasm related-epicardial coronary artery is impaired. This may play an important role in the pathogenesis of human epicardial coronary artery spasm.

REFERENCES

1. Oliva PB, Potts DE, Pluss RG. Coronary arterial spasm in Prinzmetal angina. Documentation by coronary angiography. N Engl J Med 1973; 288: 745-51.
2. Yasue H, Horio Y, Nakamura N, Fujii H, Imoto N, Sonoda R, Kugiyama K, Obata K, Morikami Y, Kimura T. Induction of coronary artery spasm by acetylcholine in patients with variant angina: possible role of the parasympathetic nervous system in the pathogenesis of coronary artery spasm. Circula-

tion 1986; 74: 955-63.
3. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986; 315: 1046-51.
4. Maseri A, Davies G, Hackett D, Kaski JC. Coronary artery spasm and vasoconstriction. The case of distinction. Circulation 1990; 81: 1983-91.
5. Yokoyama M, Akita H, Hirata K, Usuki S, Fukuzaki H, Itoh H, Maekawa K, Matsuo T. Supersensitivity of isolated coronary artery to ergonovine in a patient with variant angina. Am J Med 1990; 89: 507-15.
6. Golino P, Piscione F, Willerson JT, Cappelli-Bigazzi M, Focaccio A, Villari B, Indolfi C, Russolillo E, Condorelli M, Chiariello M. Divergent effects of serotonin on coronary-artery dimensions and blood flow in patients with coronary atherosclerosis and control patients. N Engl J Med 1991; 324: 641-8.
7. Egashira K, Tomoike H, Yamamoto Y, Yamada A, Hayashi Y, Nakamura M. Histamine-induced coronary spasm in regions of intimal thickening in miniature pigs: roles of serum cholesterol and spontaneous or induced intimal thikening. Circulation 1986; 74: 826-37.
8. Egashira K, Inou T, Yamada A, Hirooka Y, Takeshita A. Preserved endothelium-dependent vasodilation at the vasospastic site in patients with variant angina. J Clin Invest 1992; 89: 1047-52.
9. Kuga T, Egashira K, Mohri M, Tsutsui H, Harasawa Y, Urabe Y, Ando S, Shimokawa H, Takeshita A. bradykinin-induced vasodilation is impaired at the atherosclerotic site but is preserved at the spastic site of human coronary arteries in vivo. Circulation 1995; 92: 183-9.
10. Yamamoto Y, Tomoike H, Egashira K, Nakamura M. Attenuation of endothelium-related relaxation and enhanced responsiveness of vascular smooth muscle in spastic coronary artery segments from miniature pigs. Cir Res 1987; 61: 772-8.
11. Satoh S, Tomoike H, Mitsuoka W, Egashira S, Tagawa H, Kuga T, Nakamura M. Smooth muscles from spastic coronary segments show hypercontractility to histamine. Am J Physiol 1990; 259: H9-13.
12. Furchgott RF. Role of endothelium in responses of vascular smooth muscle. Cir Res 1983; 53: 557-73.
13. Kalsner S. Cholinergic mechanisms in human coronary artery preparations. Implications of special differences. J Physiol 1985; 358: 509-22.
14. Davies PF. Flow-mediated endothelial mechanotransduction. Physiological Reviews 1995; 75: 519-60.
15. Smiesko V, Kozik J, Dolezel S. Role of endothelium in the control of arterial diameter by blood flow. Blood Vessels 1985; 22: 247-51.
16. Rubanyi GM, Romero JC, Vanhotte PM. Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986; 250: H1145-9.
17. Inoue T, Tomoike H, Hisano K, Nakamura M. Endothelium determines flow-dependent dilation of the epicardial coronary

artery in dogs. *J Am Coll Cardiol 1988; 11: 187-91.*

18. Nabel EG, Selwyn AP, Ganz P. *Large coronary arteries in humans are responsive to changing blood flow: an endothelium dependent mechanism that fails in patients with atherosclerosis. J Am Coll Cardiol 1990; 16: 357-8.*

19. Celermajer DS, Sorenen KE, Catherine B, Robinson J, Deanfield JE. *Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. J Am Coll Cardiol 1994; 24: 1468-74.*

20. Gurne O, Chenu P, Buche M, Jamart J, Louagie Y, Eucher P, Marchandise B, Schroeder E. *Flow-mediated vasodilation during pacing of the free epigastric artery bypass graft early and late postoperatively. J Am Coll Cardiol 1996; 27: 415-20.*

21. Antony I, Lerebours G, Nitenberg A. *Loss of flow-dependent coronary artery dilation in patients with hypertension. Circulation 1995; 91: 1624-8.*

22. Anderson TJ, Gerhard M, Meredith IT, Charbonneau F, Delagrange D, Creager MA, Selwyn AP, Ganz P. *Systemic nature of endothelial dysfunction in atherosclerosis. Am J Cardiol 1995; 75: 71B-74B.*

23. Song JK, Park SW, Kim JJ, Doo YC, Kim WO, Park SJ, Lee SJK. *Values of intravenous ergonovine test with two-dimensional echocardiography for diagnosis of coronary artery spasm. J Am Soc Echocardiogr 1994; 7: 607-15.*

24. Song JK, Lee SJK, Kang DH, Cheong SS, Hong MK, Kim JJ, Park SW, Park SJ. *Ergonovine echocardiography as a screening test for diagnosis of vasospastic angina before coronary angiography. J Am Coll Cardiol 1996; 27: 1156-61.*

25. Yasue H, Omote S, Takizawa A, Nagao M, Milwa K, Tanaka S. *Circadian variation of exercise capacity in patients with Prinzmetal's variant angina: role of exercise-induced coronary artery spasm. Circulation 1979; 59: 938-48.*

26. Maseri A, Chierchia S. *Coronary artery spasm: demonstration, definition, diagnosis, and consequence. Prog Cardiovasc Dis 1982; 25: 169-72.*

27. Araki H, Koiwaya Y, Nakagaki O, Nakamura M. *Diurnal*

distribution of ST elevation and related arrhythmias in patients with variant angina: a study by ambulatory ECG monitoring. Circulation 1983; 67: 995-1000.*

28. Waters DD, Miller D, Bouchard A, Bosch X, Theroux P. *Circadian variation in variant angina. Am J Cardiol 1984; 54: 61-4.*

29. Ofili EO, Labovitz AJ, Kern MJ. *Coronary flow velocity dynamics in normal and diseased arteries. Am J Cardiol 1993; 71: 3D-9D.*

30. Holtz J, Forstermann U, Pohl U, Giesler M, Bassenge E. *Flow-dependent, endothelium-mediated dilation of epicardial coronary arteries in conscious dogs: effects of cyclo-oxygenase inhibition. J Cardiovasc Pharmacol 1984; 6: 1161-9.*

31. Hintze TH, Vatner SF. *Reactive dilation of large coronary arteries in conscious dogs. Cir Res 1984; 54: 50-7.*

32. el-Tamimi H, Mansour M, Pepine CJ, Wargovich TJ, Chen H. *Circadian variation in coronary tone in patients with stable angina. Protective role of endothelium. Circulation 1995; 92: 3201-5.*

33. MacAlpin RN. *Relation of coronary arterial spasm to sites of organic stenosis. Am J Cardiol 1980; 46: 143-53.*

34. Park SJ, Park SW, Song JK, Kim JJ, Kim TW, Doo YC, Kim WH, Kang DH, Lee SJK. *Clinical, angiographic characteristics and long-term follow-up in patients with variant angina. Korean Cir J 1994; 24: 349-63.*

35. Okumura K, Yasue H, Matsuyama K, Ogawa H, Kukiyama K, Ishizaka H, Sumida H, Fujii H, Matsunaga T, Tsunoda R. *Diffuse disorder of coronary artery vasomotility in patients with coronary spastic angina. Hyperreactivity to the constrictor effects of acetylcholine and the dilator effects of nitroglycerin. J Am Coll Cardiol 1996; 27: 45-52.*

36. Hackett D, Larkin S, Chierchia S, Davies G, Kaski JC, Maseri A. *Induction of coronary artery spasm by a direct local action of ergonovine. Circulation 1987; 75: 577-82.*

37. Bradley AJ, Alpert JS, Mass W. *Coronary flow reserve. Am Heart J 1991; 122: 1116-28.*