View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

© 2003 by the American College of Cardiology Foundation
Published by Elsevier Science Inc.

ISSN 0735-1097/03/$30.00
PII S0735-1097(02)02632-3

Rho-Kinase Inhibition With Intracoronary Fasudil Prevents Myocardial Ischemia in Patients With Coronary Microvascular Spasm

Masahiro Mohri, MD, PHD, Hiroaki Shimokawa, MD, PHD, Yoji Hirakawa, MD, Akihiro Masumoto, MD, Akira Takeshita, MD, PHD, FACC

Fukuoka, Japan

OBJECTIVES We sought to determine whether a potent Rho-kinase inhibitor fasudil prevents the occurrence of myocardial ischemia in patients with microvascular angina attributable to coronary microvascular spasm.

BACKGROUND Effective treatment of patients with angina who have normal coronary arteriograms (microvascular angina) has not yet been established. Rho-kinase–mediated calcium sensitization of the myosin light chain in smooth muscle cells has been implicated as substantially contributing to vascular hyperconstriction.

METHODS We studied consecutive 18 patients with angina and normal epicardial coronaries in whom intracoronary acetylcholine (ACh) induced myocardial ischemia (ischemic electrocardiographic changes, myocardial lactate production, or both) without angiographically demonstrable epicardial coronary vasospasm. All patients underwent a second ACh challenge test after pretreatment with either saline ($n = 5$) or fasudil (4.5 mg intracoronarily, $n = 13$).

RESULTS Myocardial ischemia was reproducibly induced by ACh in the saline group. In contrast, 11 of the 13 patients pretreated with fasudil had no evidence of myocardial ischemia during the second infusion of ACh ($p < 0.01$). The lactate extraction ratio (median value [interquartile range]) during ACh infusion was improved by fasudil pretreatment, from −0.16 (−0.25 to 0.04) to 0.09 (0.05 to 0.18) ($p = 0.0125$).

CONCLUSIONS Fasudil ameliorated myocardial ischemia in patients who were most likely having coronary microvascular spasm. The inhibition of Rho-kinase may be a novel therapeutic strategy for this group of patients with microvascular angina. (J Am Coll Cardiol 2003;41:15–9)
© 2003 by the American College of Cardiology Foundation

Angina with normal coronary arteriograms (cardiac syndrome X or microvascular angina) still remains a dilemma both for patients and physicians. It has posed a long-lasting health care problem, because many patients continue to have chest pain and even be disabled while taking conventional anti-anginal medications (1). Effective treatment has not been established, at least partly because it may include divergent clinical entities (2). We and others have reported that myocardial ischemia caused by abnormal microvascular constriction (spasm) might be the cause of chest pain in a subset of patients with microvascular angina (3–5). The prevention of microvascular spasm may therefore be a rational approach in these patients.

Rho-kinase modulates calcium sensitivity of the myosin light chain in smooth muscle cells (6) and has been implicated as playing a pathogenetic role in divergent cardiovascular disorders (7). Within this context, we recently demonstrated that the Rho-kinase–mediated pathway is majorly involved in the pathogenesis of epicardial coronary artery spasm in pigs (8,9) and in patients with vasospastic angina (10). In the present study, we tested the hypothesis that a Rho-kinase inhibitor might be effective in preventing myocardial ischemia in patients with microvascular angina attributable to coronary microvascular spasm.

From the Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan. This study was supported by grants from the Japanese Ministry of Science, Education, and Culture, Tokyo, Japan.

Manuscript received June 12, 2002; revised manuscript received August 7, 2002, accepted August 29, 2002.

METHODS

Patients. Eighteen patients with angina who underwent diagnostic cardiac catheterization, with a diagnosis of microvascular angina between January 1999 and December 2000, participated in the study. Clinical and angiographic features are summarized in Table 1. All patients were female and had angina at rest, on effort, or both. Seven of the 18 patients had been treated with calcium antagonists before admission; calcium antagonists were effective in two of these patients, partially effective in three, and ineffective in two. No patient had significant (>50%) organic stenosis in any major epicardial coronary artery or a history of revascularization procedures, severe valvular heart disease, idiopathic dilated or hypertrophic cardiomyopathy, or chronic renal failure. The study protocol was approved by the Institutional Ethical Committee on Human Research. We obtained written, informed consent from each patient before the study.

Study protocol. Cardiac catheterization was performed in patients in the fasting state after 5 mg oral diazepam. No patient had ever been on long-acting calcium channel blockers or hormone replacement therapy, and all cardiovascular medications, except sublingual nitroglycerin, were discontinued at least 24 h before the study. Coronary

Abbreviations and Acronyms
ACh = acetylcholine
ECG = electrocardiogram/electrocardiographic
ISDN = isosorbide dinitrate

arteriography was done by the femoral approach. A 6F pacing catheter was placed in the right ventricle to prevent bradycardia during acetylcholine (ACh) infusion. Another 6F catheter was advanced into the coronary sinus vein to sample blood for measurement of lactate concentrations.

The diagnostic protocol for coronary microvascular spasm was reported previously (5,11). Briefly, graded doses of ACh (10, 30, and 100 μg) were infused over 30 s into the left coronary artery through a 6F Judkins catheter. At 1 min after each dose of ACh was given, paired samples of 2 ml blood were collected from the coronary artery and coronary sinus vein for measurement of lactate concentrations. Biplane coronary arteriograms were then taken to assess the lumen diameter of large epicardial coronary segments. When chest pain, electrocardiographic (ECG) changes, or epicardial spasm did not occur, we gave the next dose of ACh. We considered that chest pain was caused by myocardial ischemia of microvascular origin when angina was associated with evidence of myocardial ischemia (ischemic ECG changes, myocardial lactate production, or both), but without epicardial coronary artery spasm (5,11). Epicardial coronary spasm was defined as >75% diameter reduction, compared with the diameter after intracoronary isosorbide dinitrate (ISDN) administration (5,12), and the subject who had an epicardial spasm was excluded from the study.

When ACh induced chest pain and ischemic ECG changes without angiographically demonstrable epicardial spasm, we carefully observed the patient by continuously monitoring the arterial blood pressure and 12-lead ECG and by taking a coronary arteriogram at 1-min intervals to confirm the absence of epicardial spasm. After the chest pain and ECG changes had subsided spontaneously, patients were allocated to either the group receiving saline (n = 5) or fasudil (n = 13). Saline or fasudil (300 μg/min) was infused over 15 min into the left coronary artery through the Judkins catheter. Then, the same dose of ACh that had provoked angina was infused for a re-challenge. After the conclusion of the study, ISDN was administered into the left coronary artery.

Drugs. We used the following drugs: fasudil (Asahi Chemical Industries, Tokyo), ACh (Daiichi-Seiyaku, Tokyo), and ISDN (Eisai, Tokyo). All drugs were diluted in physiologic saline immediately before use. We have recently demonstrated that with our dosing protocol of fasudil (300 μg/min for 15 min intracoronarily), the concentrations of fasudil were raised to 3.7 ± 0.4 (SD) μmol/l in the coronary circulation in patients without obstructive coronary artery disease (10). This value is higher than the reported half-maximal inhibitory concentration (IC_{50}) of fasudil for Rho-kinase inhibition (1.8 to 1.9 μmol/l) (13).

Measurements. Quantitative coronary arteriography was performed with a Siemens biplane cineangiographic system (Bicor/Hicor, Siemens, Erlangen, Germany), as reported (14,15). Measurements were taken at 10 segments of the left coronary artery: left main trunk; proximal, middle, and distal segments of the left anterior descending coronary artery; first and second diagonal branches; proximal and distal segments of the left circumflex artery; obtuse marginal branch; and posterolateral branch. Segments that showed the greatest constrictive response in the left anterior descending coronary artery and left circumflex artery were used for analysis. The degree of vasoconstriction was normalized by the diameter obtained after ISDN administration and was presented as the percent diameter reduction.

Electrocardiographic changes were considered ischemic when transient ST-segment depression or elevation >0.1 mV at 80 ms after the J point was noted in at least two leads. Lactate concentrations of sampled blood were immediately determined with a lactate analyzer (2300 Stat Plus, YSI, Yellow Springs, Ohio). The myocardial lactate extraction ratio was calculated as the ratio of the coronary arterial-venous difference in lactate concentration to the arterial concentration. Myocardial lactate production (i.e., negative extraction ratio) was considered to be evidence of myocardial ischemia.

Statistics. Data are presented as the median value (interquartile range). Comparisons of continuous variables were done by using the Wilcoxon signed-rank test. Effects of treatment (saline vs. fasudil) on the prevalence of angina and myocardial ischemia during ACh infusion were analyzed by the chi-square test. A p value <0.05 was considered statistically significant.

Table 1. Clinical and Angiographic Features of the 18 Study Patients

Age (yrs)	64 (57–70)
Angina	
Rest	7 (39%)
Effort	3 (17%)
Rest and effort	8 (44%)
Angina >30 min	7 (39%)
Positive exercise ECG	4 (22%)
Coronary risk factors	
Hypertension	6 (33%)
Diabetes mellitus	1 (6%)
Current or past smoking	0 (0%)
Hypercholesterolemia	4 (22%)
Total cholesterol (mg/dl)	197 (181–208)
LDL cholesterol (mg/dl)	117 (97–133)
Maximal diameter stenosis* (%)	0 (0–19)

*Measured after administration of isosorbide dinitrate. Data are presented as the median value (interquartile range) or number (%) of patients.
ECG = electrocardiogram; LDL = low-density lipoprotein.

Table 2. Prevalence of Angina and Ischemic Electrocardiographic Changes, Myocardial Lactate Metabolism, Systemic Hemodynamics, and the Degree of Epicardial Constriction at Acetylcholine Testings

	Saline (n = 5)		Fasudil (n = 13)	
	First ACh	Second ACh	First ACh	Second ACh
Angina	5 (100%)	5 (100%)	13 (100%)	4 (31%)‡
ECG changes	5 (100%)	4 (80%)	12 (92%)	2 (15%)§
Lactate production*	5 (100%)	5 (100%)	8 (80%)	2 (20%)‡
Lactate extraction ratio	−0.13 (−0.33 to −0.09)	−0.19 (−0.42 to −0.06)	−0.16 (−0.25 to 0.04)	0.09 (0.05 to 0.18)¶
Epicardial constriction (%)†				
LAD	−18 (−34 to −14)	−26 (−30 to −17)	−20 (−40 to −13)	−11 (−32 to −7)
LCx	−23 (−24 to −21)	−17 (−23 to −14)	−19 (−26 to −11)	−13 (−21 to −7)

*Not determined in three patients in the fasudil group. †Expressed as the percent reduction in lumen diameter from that after nitrate administration. ‡$p < 0.05$. §$p < 0.01$ for saline vs. fasudil (by the chi-square test). ¶$p = 0.013$ for first ACh vs. second ACh (by the Wilcoxon signed-rank test). Data are shown as the number (%) of patients or median value (interquartile range).

ACh = acetylcholine; ECG = electrocardiogram; LAD = left anterior descending coronary artery; LCx = left circumflex artery.

RESULTS

Acetylcholine challenge testing. During the first ACh test, all 18 patients had chest pain and evidence of myocardial ischemia without epicardial spasm (Table 2, Figs. 1 and 2).

In the saline group (n = 5), both angina and myocardial lactate production were reproducibly induced by the second ACh challenge test. The myocardial lactate extraction ratio during the first and second ACh infusions was unchanged (−0.13 [−0.33 to −0.09] vs. −0.19 [−0.42 to −0.06]; p = NS). In the fasudil group (n = 13), the second ACh challenge did not provoke myocardial ischemia in 11 patients ($p < 0.01$, saline vs. fasudil), although two of the 11 patients had chest pain. The remaining two patients had anginal pain, ischemic ECG changes, and lactate produc-

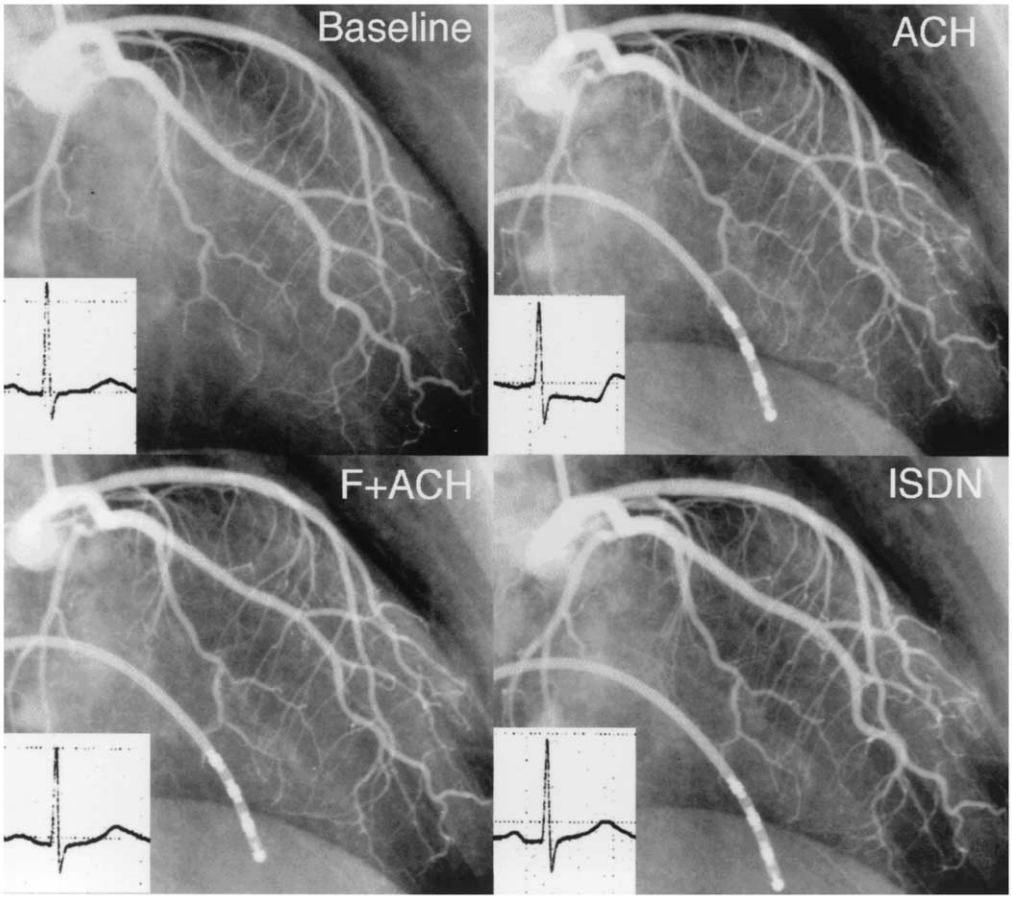

Figure 1. Representative coronary arteriograms and electrocardiographic (ECG) tracings (lead V_4) of a patient from the fasudil group. **(Top left)** Baseline coronary arteriogram shows a normal left coronary artery. **(Top right)** Acetylcholine (ACh) induced angina and downsloping ST-segment depression, but no epicardial spasm. Lactate production was also noted. **(Bottom left)** The second ACh test after pretreatment with fasudil (F) did not provoke angina, ischemic ECG changes, epicardial spasm, or lactate production. **(Bottom right)** Normal coronary arteriograms after administration of isosorbide dinitrate.

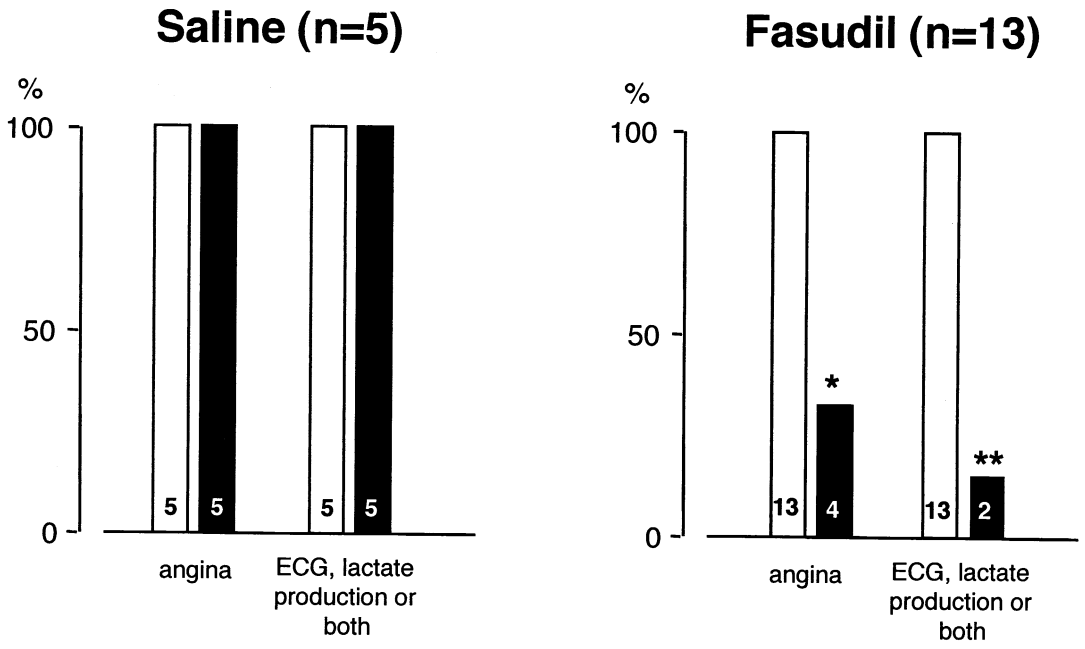

Figure 2. Bar graphs showing the incidence of angina and evidence of myocardial ischemia (ischemic electrocardiographic [ECG] changes, lactate production, or both) during the first **(open bars)** and second **(solid bars)** acetylcholine challenges. *$p < 0.05$, **$p < 0.01$, saline vs. fasudil (by the chi-squared test).

tion during ACh infusion, even after pretreatment with fasudil. Overall, fasudil pretreatment significantly improved the myocardial lactate extraction ratio from −0.16 [−0.25 to 0.04] to 0.09 [0.05 to 0.18] in the Fasudil group ($p = 0.0125$ by the Wilcoxon signed-rank test).

Hemodynamics and epicardial diameters. Systolic arterial pressure and heart rate were comparable at the first and second ACh administrations, both in the saline and fasudil groups (data not shown). The magnitude of ACh-induced epicardial coronary vasoconstriction was minimal and did not differ between the first and second ACh challenges (Table 2).

DISCUSSION

We demonstrated that intracoronary fasudil prevented ACh-induced angina and myocardial ischemia in patients with coronary microvascular spasm. Because this effect was not associated with changes in systemic blood pressure, heart rate, or the magnitude of epicardial coronary constriction, it is suggested that fasudil suppressed ACh-induced coronary microvascular hyperconstriction in our patients.

Mechanism of myocardial ischemia in microvascular angina. The effective treatment of microvascular angina has not been established, and a substantial proportion of patients remain symptomatic even while taking conventional anti-anginal medications (16–18). Recently, we reported that coronary microvascular spasm and resultant myocardial ischemia might be the cause of chest pain in a subgroup of these patients (5). We have shown that angina was associated with myocardial lactate production, definite evidence of myocardial ischemia, but without epicardial coronary artery hyperconstriction. Constrictive responses of epicardial segments to ACh were minimal (<25%) in our patients, suggesting that myocardial ischemia was caused primarily by coronary microvascular spasm.

Rho-kinase as a therapeutic target. To suppress microvascular spasm, we have targeted Rho-kinase for the following reasons. First, it has been shown that Rho-kinase-mediated phosphorylation of myosin phosphatase plays a central role in smooth muscle hypercontraction (6,7,19). Second, we have demonstrated that a Rho-kinase inhibitor such as fasudil and Y-27632 prevented *epicardial* coronary vasospasm in the animal model (8,9) and in patients with vasospastic angina (10). Third, we have recently shown that Rho-kinase was involved in increased tone of peripheral *resistance* vessels in hypertensive patients (20).

Fasudil has been shown to be a selective and potent Rho-kinase inhibitor when tested in vitro and in animals (8,13,21). As already mentioned in the Methods section, our dosing protocol raised the concentrations of fasudil to 3.7 μmol/l in the coronary circulation (10), which is higher than the reported IC_{50} of fasudil for Rho-kinase inhibition (1.8 to 1.9 μmol/l) (13,22). In addition, we previously demonstrated that a comparable dose of intracoronary Fasudil inhibited the activity of myosin phosphatase, a target protein of Rho-kinase, in pigs in vivo (23). These lines of evidence suggest that the beneficial effect of fasudil observed in the present study was brought about, in large part, through the inhibition of Rho-kinase. Intriguingly, abnormal hypercontraction of coronary microvessels has been suggested to contribute to myocardial ischemia also in patients with epicardial coronary artery disease (24,25).

Whether the Rho-kinase inhibitor is effective in ameliorating myocardial ischemia in these patients remains to be examined. Finally, it should be noted that patients enrolled in the present study had objective evidence of myocardial ischemia (i.e., lactate production). Therefore, our results do not necessarily suggest a general use of Rho-kinase inhibitors in patients with chest pain and normal epicardial coronaries, but no evidence of myocardial ischemia.

Study limitations. First, coronary microvascular spasm was not angiographically documented in our patients, and its contribution to myocardial ischemia was only indirectly suggested. However, as discussed earlier, it seems very unlikely that other factors, such as epicardial constriction or hemodynamic alterations, played a primary role in the development of myocardial ischemia during ACh infusion. Second, the long-term effect of Rho-kinase inhibition in these patients is unknown. An orally active preparation of fasudil is now under investigation and will be available in the near future (26). Thus, trials are warranted to determine whether oral fasudil improves the anginal status and/or the quality of life in patients with angina caused by coronary microvascular spasm.

Conclusions. Fasudil was effective in preventing ACh-induced myocardial ischemia in patients with angina most likely caused by hyperconstriction of coronary microvessels. We suggest that inhibition of Rho-kinase may be a novel therapeutic strategy for patients with microvascular angina in whom coronary microvascular spasm is causatively involved.

Reprint requests and correspondence: Dr. Masahiro Mohri, Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka 812-8582, Japan. E-mail: mmohri@med.kyushu-u.ac.jp.

REFERENCES

1. Kaski JC, Rosano GMC, Collins P, Nihoyannopoulos P, Maseri A, Poole-Wilson PA. Cardiac syndrome X: clinical characteristics and left ventricular function. A long-term follow-up study. J Am Coll Cardiol 1995;25:807–14.
2. Kaski JC, Elliott PM. Angina pectoris and normal coronary arteriograms: clinical presentation and hemodynamic characteristics. Am J Cardiol 1995;76:35D–42D.
3. Cannon RO, Cunnion RE, Parrillo JE, Palmeri ST, Schenke WH, Epstein SE. Dynamic limitation of coronary vasodilator reserve in patients with dilated cardiomyopathy and chest pain. J Am Coll Cardiol 1987;10:1190–200.
4. Clarke JG, Davies GJ, Kerwin R, et al. Coronary artery infusion of neuropeptide Y in patients with angina pectoris. Lancet 1987;1: 1057–9.
5. Mohri M, Koyanagi M, Egashira K, et al. Angina pectoris caused by coronary microvascular spasm. Lancet 1998;351:1165–9.
6. Somlyo AP, Somlyo AV. Signal transduction by G-proteins, rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. J Physiol 2000;522:177–85.
7. Shimokawa H. Rho-kinase as a novel therapeutic target in treatment of cardiovascular diseases. J Cardiovasc Pharmacol 2002;39:319–27.
8. Shimokawa H, Seto M, Katsumata N, et al. Rho-kinase–mediated pathway induces enhanced myosin light chain phosphorylations in a swine model of coronary artery spasm. Cardiovasc Res 1999;43:1029–39.
9. Kandabashi T, Shimokawa H, Miyata K, et al. Inhibition of myosin phosphatase by upregulated Rho-kinase plays a key role for coronary artery spasm in a porcine model with interleukin-1β. Circulation 2000;101:1319–23.
10. Masumoto A, Mohri M, Shimokawa H, Urakami L, Usui M, Takeshita A. Suppression of coronary artery spasm by the Rho-kinase inhibitor Fasudil in patients with vasospastic angina. Circulation 2002;105:1545–7.
11. Sun H, Mohri M, Shimokawa H, Usui M, Urakami L, Takeshita A. Coronary microvascular spasm causes myocardial ischemia in patients with vasospastic angina. J Am Coll Cardiol 2002;39:847–51.
12. Sugiishi M, Takatsu F. Cigarette smoking is a major risk factor for coronary spasm. Circulation 1993;87:76–9.
13. Davies SP, Reddy H, Caivano M, Cohen P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem J 2000;351:95–105.
14. Kuga T, Egashira K, Mohri M, et al. 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.
15. Tagawa T, Mohri M, Tagawa H, et al. Role of nitric oxide in substance P–induced vasodilation differs between the coronary and forearm circulation in humans. J Cardiovasc Pharmacol 1997;29:546–53.
16. Kaski JC, Valenzuela Garcia LF. Therapeutic options for the management of patients with cardiac syndrome X. Eur Heart J 2001;22: 283–93.
17. Lanza GA, Colonna G, Pasceri V, Maseri A. Atenolol versus amlodipine versus isosorbide-5-mononitrate on anginal symptoms in syndrome X. Am J Cardiol 1999;84:854–6.
18. Masumoto A, Mohri M, Takeshita A. Three-year follow-up of the Japanese patients with microvascular angina attributable to coronary microvascular spasm. Int J Cardiol 2001;81:151–6.
19. Uehata M, Ishizaki T, Satoh H, et al. Calcium sensitization of smooth muscle mediated by a rho-associated protein kinase in hypertension. Nature 1997;389:990–4.
20. Masumoto A, Hirooka Y, Shimokawa H, Hironaga K, Setoguchi S, Takeshita A. Possible involvement of Rho-kinase in the pathogenesis of hypertension in humans. Hypertension 2001;38:1307–10.
21. Swärd K, Dreja K, Susnjar M, Hellstrand P, Hartshorne DJ, Walsh MP. Inhibition of rho-associated kinase blocks agonist-induced Ca^{2+} sensitization of myosin phosphorylation and force in guinea-pig ileum. J Physiol 2000;522:33–49.
22. Batchelor TJ, Sadaba JR, Ishola A, Pacaud P, Munsch CM, Beech DJ. Rho-kinase inhibitors prevent agonist-induced vasospasm in human internal mammary artery. Br J Pharmacol 2001;132:302–8.
23. Katsumata N, Shimokawa H, Seto M, et al. Enhanced myosin light chain phosphorylations as a central mechanism for coronary artery spasm in a swine model with interleukin-1β. Circulation 1997;96: 4357–63.
24. Golino P, Pisgione F, Benedict CR, et al. Local effect of serotonin released during coronary angioplasty. N Engl J Med 1994;330:523–8.
25. Sambuceti G, Marzilli M, Marraccini P, et al. Coronary vasoconstriction during myocardial ischemia induced by rises in metabolic demand in patients with coronary artery disease. Circulation 1997;95:2652–9.
26. Shimokawa H, Hiramori K, Iinuma H, et al. Antianginal effect of Fasudil, a Rho-kinase inhibitor, in patients with stable effort angina: a multicenter study. J Cardiovasc Pharmacol 2002;40:751–61.