Suppression of Coronary Artery Spasm by the Rho-Kinase Inhibitor Fasudil in Patients With Vasospastic Angina

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Background—Increased activity of Rho-kinase causes hypercontraction of vascular smooth muscle and has been implicated as playing a pathogenetic role in divergent cardiovascular diseases such as coronary artery spasm. We examined whether an intracoronary infusion of fasudil, a selective Rho-kinase inhibitor, would attenuate coronary vasoconstrictor responses to acetylcholine (ACh) in patients with vasospastic angina.

Methods and Results—We studied 20 consecutive patients in whom coronary artery spasm was provoked by intracoronary ACh. The patients underwent a second ACh challenge after pretreatment with intracoronary saline (n=5) or fasudil (n=15; 300 μg/min for 15 minutes). Angina and coronary vasospasm were reproducibly induced by the second testing in patients who received saline. In contrast, fasudil markedly attenuated the coronary constriction induced by ACh (P<0.001) and prevented the occurrence of chest pain and ischemic ECG changes in all treated patients (both P<0.01 versus saline). Fasudil, at the dose used in this study, did not significantly change systemic hemodynamics or baseline coronary blood flow.

Conclusions—Fasudil was effective in preventing ACh-induced coronary artery spasm and resultant myocardial ischemia in patients with vasospastic angina. We suggest that this Rho-kinase inhibitor may be a novel therapeutic intervention to treat ischemic coronary syndromes caused by coronary artery spasm.

Key Words: angina • ischemia • circulation

Coronary artery spasm is the underlying mechanism in a broad spectrum of ischemic heart diseases, including Prinzmetal’s variant angina, unstable angina, acute myocardial infarction, and sudden cardiac death.1 Recently, we demonstrated that increased activity of the Rho-kinase-mediated pathway in vascular smooth muscle plays a central role in the genesis of enhanced vasoconstriction in animal models of hypertension2 and coronary artery spasm.3,4 However, it remains to be determined whether the inhibition of this Rho-kinase pathway will provide a novel therapeutic approach in patients with vasospastic disorders. Thus, in the present study, we tested our hypothesis that pretreatment with intracoronary fasudil, a potent Rho-kinase inhibitor, will inhibit coronary vasoconstritor responses to acetylcholine (ACh) and prevent myocardial ischemia in patients with vasospastic angina.

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Methods

Patients

We studied 20 consecutive patients (10 men and 10 women; mean age, 62 years; range, 49 to 74 years) with rest angina who underwent coronary arteriography and had a positive ACh challenge.

Drugs

We used the following drugs: fasudil (Asahi Chemical Industries), ACh (Daiichi-Seiyaku), and isosorbide dinitrate (Eisai). All drugs were diluted in physiological saline immediately before use.

Study Protocols

The study protocol was approved by the Institutional Ethics Committee on Human Research, and we obtained written informed consent from each patient before the study.

The protocol of our spasm provocation testing was reported previously.5,6 Briefly, we infused graded doses of ACh (10, 30, and 100 μg) into the left coronary artery. Coronary artery spasm was defined as >75% diameter reduction compared with the diameter after the infusion of intracoronary isosorbide dinitrate at one or more epicardial segments.5–7 After the induction of spasm, we carefully followed the patient by continuously monitoring arterial pressure and 12-lead ECG and by taking serial coronary arteriograms at 1-minute intervals. Within a few minutes after ACh infusion, coronary spasm and ischemic ECG changes spontaneously subsided without nitrates or any other treatment. Thereafter, either saline (n=5) or fasudil (n=15; 300 μg/min) was infused over 15 minutes into the left coronary artery via a Judkins catheter, and the same dose of ACh that had induced the spasm was infused for re-challenge. For ethical reasons, subjects with a total or subtotal vasospastic occlusion were prospectively excluded from the study, as were patients who had severe chest pain, hypotension, or both; these patients were immediately treated with intracoronary isosorbide dinitrate.
Measurements
Quantitative coronary arteriography was performed with a validated densitometric analysis system, as previously reported.8,9 Coronary luminal diameter was measured at both the spastic and nonspastic segments at proximal portions of the left anterior descending coronary artery and the left circumflex coronary artery. Constrictor responses to ACh were quantified as percent reduction from the luminal diameter obtained after the administration of isosorbide dinitrate. Standard 12-lead ECG and systemic arterial pressure were continuously monitored throughout the study. To determine the effect of fasudil on baseline coronary blood flow, we measured coronary flow velocity in the left anterior descending coronary artery with a 0.014-inch Doppler guidewire (FloWire, Cardiometrics) during the drug infusion.10 The wire was withdrawn before the administration of ACh to avoid vascular injury in case spasm was provoked. In the first 6 patients allocated to the fasudil group, coronary sinus venous blood was sampled at the end of drug infusion to determine the concentration of fasudil.11

Statistics
Data are shown as mean±SD. Comparison of continuous and discrete variables was performed by the t test and \( \chi^2 \) test, respectively. Two-way ANOVA was used to compare vessel diameter responses between the 2 groups treated with saline and fasudil. \( P<0.05 \) was considered statistically significant.

Results
ACh provoked coronary artery spasm in all 20 patients and was associated with chest pain (n=19) and ischemic ECG changes (n=18; Figure 1). In the 5 patients who received saline, angina and ischemic ECG changes were reproducibly induced by the second ACh challenge. Constrictor responses were also comparable during the first and second ACh infusion, both at the spastic and nonspastic segments (Figure 2). In contrast, no patient treated with fasudil (n=15) developed chest pain or ischemic ECG changes during the second ACh challenge (both \( P<0.01 \) versus saline). Vasoconstrictor responses to ACh were markedly attenuated with fasudil pretreatment at the segments where coronary vasospasm had been provoked during the first ACh test (\( P<0.001 \) by 2-way ANOVA for treatment; Figures 1 and 2). Intriguingly, the magnitude of constriction at the nonspastic segments was not significantly different before and after the treatment with fasudil (Figure 2B).

Fasudil did not change baseline systemic arterial pressure or heart rate (data not shown). Coronary blood flow before and after the 15-minute infusion of fasudil was 82±38 mL/min and 90±60 mL/min, respectively (\( P=NS \)). The concentrations of fasudil in the coronary sinus venous blood increased to 3.7±0.4 \( \mu \)mol/L immediately after the 15-minute infusion (n=6). No complication such as hypotension, hemorrhage, or myocardial infarction occurred in any of the patients.

Discussion
To the best of our knowledge, this is the first study that demonstrates that the Rho-kinase inhibitor fasudil is effective in suppressing coronary artery spasm and the resultant myocardial ischemia in patients with vasospastic angina. Rho-kinase reduces myosin phosphatase activity by phosphorylating the myosin-binding subunit of the enzyme and thus augments vascular smooth muscle contraction at a given calcium concentration, which is known as “calcium sensitization.”12 Fasudil is a potent and selective inhibitor of Rho-kinase,13–16 with its inhibitory effect on Rho-kinase being ~10 times and 100 times more potent than on protein kinase C and myosin light chain kinase, respectively. Although the tissue concentrations of fasudil are not known in our patients, we measured fasudil concentrations in the coronary circulation. We and others have previously shown that the IC50 value of fasudil is \(<1.9 \mu \text{mol/L} \) when tested in vitro,14,15 and the achieved concentration (3.7 \( \mu \text{mol/L} \) in our patients therefore seems to be high enough to inhibit Rho-kinase activity. Indeed, a comparable dose of intracoronary fasudil (100 \( \mu \text{g/kg} \)) suppressed coronary artery spasm.
in our porcine model in vivo.\textsuperscript{14,17} In that model, it was shown that inactivation of the myosin-binding subunit of myosin light chain phosphatase, which is a major target protein of Rho-kinase, was a primary mechanism whereby fasudil or Y27632 prevented coronary artery spasm.\textsuperscript{18}

Interestingly, the effect of fasudil on ACh-mediated vasoconstriction at the nonspastic segments was minimal in our patients. This finding is in accordance with the hypothesis that the calcium sensitization of vascular smooth muscle cells mediated by the activated Rho-kinase pathway plays a key role in coronary artery spasm.\textsuperscript{3,4} Compared with calcium-channel blockers, this Rho-kinase inhibitor may be a more suitable choice for patients with vasospastic angina because of its selective spasmolytic effect on vascular segments that exhibit hypercontraction. In this context, we have recently shown that Rho-kinase is involved in the pathogenesis of increased systemic vascular resistance in hypertensive patients but not in control normotensive subjects.\textsuperscript{18} Furthermore, we also demonstrated that Rho-kinase is substantially involved in agonist-induced contractions of arteriosclerotic human internal thoracic arteries.\textsuperscript{19}

In conclusion, fasudil was highly effective in suppressing coronary artery spasm in patients with vasospastic angina. We suggest that the inhibition of Rho-kinase is a novel therapeutic strategy for treating patients with ischemic coronary syndromes caused by coronary artery spasm.

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**References**

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