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

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METHODS

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.

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.

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)

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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.

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

| Age (yrs) | 64 (57–70) |
| Angina | 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.
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-

### Table 2

<table>
<thead>
<tr>
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<th>Saline (n = 5)</th>
<th>Fasudil (n = 13)</th>
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<tbody>
<tr>
<td></td>
<td>First ACh</td>
<td>Second ACh</td>
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<tr>
<td>Angina</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>ECG changes</td>
<td>5 (100%)</td>
<td>4 (80%)</td>
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<tr>
<td>Lactate production*</td>
<td>-0.13 (-0.33 to -0.09)</td>
<td>-0.19 (-0.42 to -0.06)</td>
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<tr>
<td>Lactate extraction ratio</td>
<td>-0.13 (-0.33 to -0.09)</td>
<td>-0.19 (-0.42 to -0.06)</td>
</tr>
<tr>
<td>Epicardial constriction (%)†</td>
<td>LAD -18 (-34 to -14)</td>
<td>-26 (-30 to -17)</td>
</tr>
<tr>
<td></td>
<td>LCx -23 (-24 to -21)</td>
<td>-17 (-23 to -14)</td>
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*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.
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).

**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).
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.

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**REFERENCES**