A Comparative Evaluation of the Effects of Multiple Vasodilators on Human Internal Mammary Artery

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Background: Vasospasm of arterial grafts represents an unpredictable complication of coronary artery surgery and may compromise myocardial revascularization, and treatment is based on empirical therapy with nitroglycerin. Because of the potential for tolerance to nitroglycerin to occur, the authors studied different vasodilators acting through separate pathways on segments of human internal mammary artery.

Methods: Isolated vascular rings were precontracted with norepinephrine (1 μM), KCl, or the thromboxane A۲ analogue (U46619, 10 μM). Nitroglycerin (a nitrovasodilator), milrinone (a type III phosphodiesterase inhibitor), papaverine (a phosphodiesterase inhibitor), prostaglandin E۱, and isradipine (a dihydropyridine calcium channel blocker) were added in a cumulative fashion.

Results: The analysis of the concentration–response curves showed that vasodilators induced 90–100% relaxation of the constricted segments with norepinephrine or the thromboxane A۲ analogue, except prostaglandin E۱, which produced 75% relaxation at maximal concentrations. The effective concentrations of vasodilator agent that caused 50% relaxation for nitroglycerin and milrinone were within the range of the reported therapeutic concentrations in plasma. Isradipine was also effective at reversing receptor-mediated contraction (maximal relaxation = 100% in internal mammary artery contracted with norepinephrine; maximal relaxation = 90% in internal mammary artery contracted with thromboxane A۲ analogue).

Conclusions: Vasodilator drugs acting through multiple pathways are effective at reversing in vitro vasocstriction. (Key words: Isradipine; milrinone; nitroglycerin; norepinephrine; papaverine; prostaglandin E۱; thromboxane A۲).

THE internal mammary artery (IMA) is widely used as a conduit for coronary artery surgery. Numerous clinical studies have demonstrated the superiority of the IMA regarding short- and long-term potency compared with the saphenous vein. Further, endothelium-dependent relaxations are greater in the IMA than in the saphenous vein, and the vasoconstrictive tone of the IMA has been described to be not as potent as in other arterial conduits. It is noteworthy that gastrocnilioic and other grafts including radial arteries have not been uniformly effective as arterial grafts because of their vasoplastic properties. Vasospasm does occur during IMA grafting, however, and may compromise myocardial perfusion. The pathophysiology of this vasoconstriction is complex and includes mechanical, physical, and pharmacologic stimulations. Platelet activation and release of thromboxane A۲ may be important causal factors. In addition, the reversal of the vasospasm is often challenging, and the most effective therapy is not well defined. In vitro studies have described separately the efficacy of nitrovasodilator drugs, calcium channel blockers, and phosphodiesterase inhibitors on the IMA, but there are few data comparing different pharmacologic classes of drugs. Therefore, we investigated the effects of different classes of vasodilator drugs on human IMA precontracted with a thromboxane A۲ analogue or with norepinephrine.

Methods

Vessels Preparation

Segments of right and left IMA were collected from 60 patients undergoing coronary artery bypass surgery. The discarded distal end was removed carefully and placed in chilled modified Krebs HEPES buffer of the following composition (in mmol/L): NaCl 118, KCl 4.69, CaCl۲ 3.55, MgSO۴ 1.04, NaHCO۳ 25, D-glucose 11.1, and HEPES 21.8, pH 7.40 ± 0.05. The vessels were transferred to the laboratory and then cleaned of adher-
ent connective tissue. The time delay between vessel harvest and preparation was <15 min. The IMA segments were cut into 3-mm ring segments. One to six rings were obtained from each vessel.

Experiments with Isolated Vascular Rings
The rings were suspended between two wire hooks in organ chambers filled with 25 ml Krebs-Henseleit solution (37°C, pH 7.40) aerated with 95% O₂/5% CO₂. The upper hook was connected to a force transducer (Kent-Scientific Corporation, Litchfield, CT), and changes in isometric force were recorded (MacLab® system, ADI Instruments, Milford, MA). A resting tension (4 g) initially defined by preliminary studies was progressively applied, and the rings were allowed to stabilize for 45 min. For the contraction experiments, cumulative concentration–response curves were obtained with KCl, norepinephrine, and the thromboxane A₂ analogue. Increasing concentrations of the thromboxane A₂ analogue and norepinephrine were added to the organ bath in 0.5 log unit steps and in 0.2 log unit steps for KCl. For the relaxation studies, the rings were precontracted with norepinephrine (1 μM) or the thromboxane A₂ analogue (10 nM). The concentrations were determined from the cumulative contraction–response curves to achieve 50–80% of the maximum contraction. Segments of IMA were allowed another equilibration period of 15 min and then exposed to increasing concentrations (in 0.5 log unit steps) every 15 min using vasodilator agents, which included nitroglycerin, milrinone, papaverine, prostaglandin E₁, and isradipine.

Drugs
The following drugs were used: a thromboxane A₂ analogue (U46619) and prostaglandin E₁, (provided by Upjohn Company, Kalamazoo, MI); norepinephrine (ampules from Abbott Laboratories, Chicago, IL); and nitroglycerin (ampules from Solopack Laboratories, Elk Grove Village, IL). Papaverine and KCl were obtained from Sigma Chemical Company (St. Louis, MO), and isradipine was a gift from Sandoz Pharmaceutical (East Hanover, NJ). The thromboxane A₂ analogue was diluted in ethanol (95%) to 1 mM and then serially diluted in distilled water. Isradipine was diluted in ethanol, propylene glycol, and distilled water to a concentration of 20 mM and then serially diluted using ethanol/H₂O (40:60). The final concentration of ethanol in the bath did not exceed 0.8%. Additional experiments showed that ethanol has a slight vasoconstrictive effect on the IMA at concentrations >1.5% in the organ bath. Prostaglandin E₁ was dissolved in ethanol to reach a concentration of 30 mM and then serially diluted in distilled water. Nitroglycerin was serially diluted in distilled water. Drugs were prepared before each experiment and stored on ice. The concentrations of the drugs are expressed as final molar concentrations in the bath solution.

Data and Statistical Analysis
Contraction responses to KCl, norepinephrine, and the thromboxane A₂ analogue were expressed in gain of tension (in grams). Relaxation responses were calculated as a percentage of norepinephrine or the thromboxane A₂ analogue–induced contraction. Data are averaged for each patient in all experiments. The effective concentration of vasodilator agent that caused 50% of relaxation (EC₅₀) was determined for each IMA (responses from vascular segments were averaged for one IMA) by the logistic curve fitting the equation: E = (Eₘₐₓ × Cγ)/(C₀ + EC₅₀)γ), where E is the response, Eₘₐₓ is the maximal relaxation, C is the concentration, and γ is the slope parameter.

Results
As shown in figure 1 and table 1, the IMA segments exhibited greater contraction in the presence of the thromboxane A₂ analogue (6.4 ± 0.5 g) compared with norepinephrine (4.9 ± 0.5 g) and KCl (4.1 ± 0.7 g). The difference is significant between KCl and the thromboxane A₂ analogue and between norepinephrine and the thromboxane A₂ analogue. Nitroglycerin, papaverine, milrinone, and isradipine allowed 90–100% of the maximum relaxation of the IMA segments contracted with norepinephrine (fig. 2) or the thromboxane A₂ analogue (fig. 3). Nitroglycerin was more potent in relaxing vessels contracted with norepinephrine than vessels contracted with the thromboxane A₂ analogue.
Constriction (grams)

Fig. 1. Contraction–response curves for the thromboxane A$_2$ analogue U46619 (filled circles), norepinephrine (filled squares), and KCl (filled triangles) in human internal mammary artery. Symbols represent data averaged for five patients. Data are expressed in gain of tension (grams, mean ± SD).

(EC$_{50}$ = 0.9 ± 0.6 vs 24.6 ± 14.9 nm, respectively; $P < 0.01$). There was no difference for milrinone, papaverine, and isradipine. The EC$_{50}$ values calculated for the different vasodilators are shown in table 2 (IMA precontracted with norepinephrine) and table 3 (IMA precontracted with the thromboxane A$_2$ analogue). We noted that prostaglandin E$_1$ only partially reversed thromboxane A$_2$-induced contraction compared with nitroglycerin (73 ± 6% vs 94 ± 2% respectively; $P < 0.01$).

**Discussion**

The current study shows that nitroglycerin, milrinone, papaverine, and isradipine effectively reversed thromboxane A$_2$ analogue–induced contraction of IMA segments, although prostaglandin E$_1$ was ineffective. These drugs are clinically used and represent the major different classes of vasodilators, as shown in table 4. We also found that nitroglycerin is the most potent drug for reversing precontracted IMA with the thromboxane

**Table 1. Maximal Responses to Vasoconstrictor Drugs in IMAs**

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>$E_{max}$ (g)</th>
<th>Concentration (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCL</td>
<td>5</td>
<td>4.1 ± 1.6</td>
<td>$10^{-1.1}$</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>5</td>
<td>4.9 ± 0.5</td>
<td>$10^{-5.5}$</td>
</tr>
<tr>
<td>U46619</td>
<td>5</td>
<td>6.3 ± 1.2</td>
<td>$10^{-6}$</td>
</tr>
</tbody>
</table>

Data are mean ± SD; n is the number of patients.

U46619 = the thromboxane A$_2$ analogue; $E_{max}$ = the maximal contraction, expressed in gain of tension (g).

Anesthesiology. V 88, No 6, Jun 1998

Fig. 2. Concentration–response curves for nitroglycerin (n = 18), milrinone (n = 19), papaverine (n = 19), and isradipine (n = 12) in human internal mammary artery contracted with norepinephrine (1 µM). Data are expressed as mean ± SD; n = number of vessel segments.

Fig. 3. Concentration–response curves for nitroglycerin (n = 24), milrinone (n = 23), papaverine (n = 19), prostaglandin E$_1$ (n = 26), and isradipine (n = 24) in human internal mammary artery contracted with the thromboxane A$_2$ analogue (U46619, 10 ns). Data are expressed as mean ± SD; n = number of vessel segments.
Table 2. Effects of Different Vasodilator Agents in IMAs Precontracted with Norepinephrine

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>EC50 (nM)</th>
<th>P Value*</th>
<th>Emax (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroglycerin</td>
<td>6</td>
<td>8.8 ± 7.4 x 10^-10</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Milrinone</td>
<td>6</td>
<td>9.9 ± 7.7 x 10^-9</td>
<td>&lt;0.01</td>
<td>100</td>
</tr>
<tr>
<td>Papaverine</td>
<td>7</td>
<td>1.4 ± 1.2 x 10^-6</td>
<td>&lt;0.01</td>
<td>100</td>
</tr>
<tr>
<td>Isradipine</td>
<td>6</td>
<td>1.2 ± 1.8 x 10^-8</td>
<td>&lt;0.01</td>
<td>100</td>
</tr>
</tbody>
</table>

Data are mean ± SD; n is the number of patients.

EC50 = the concentration that caused 50% relaxation of norepinephrine-induced constriction; Emax = the maximal relaxation.

* The P value defines the difference between the EC50 of nitroglycerin and the EC50 of the other vasodilator drugs.

A1 analogue or with norepinephrine. Therapeutic concentrations of nitroglycerin in plasma range from 1–20 nM.15 The in vitro evaluation of the effects of nitrates, however, shows that they are extremely variable according to the regional vascular bed and the constrictive pharmacologic stimulation.16 Further, the concentration of nitrovasodilator agents in plasma may not be related to the therapeutic effect because the drugs are inactive in their parent molecule.17 The EC50 values for the relaxant effects of milrinone (1 μM for norepinephrine and 3 μM for thromboxane A2 analogue-induced constriction) were also almost included in the range of the therapeutic concentrations in plasma reported in patients (0.5–2.0 μM).18 For prostaglandin E1, the therapeutic concentrations in plasma are unknown. Papaverine is only used in intra- and extraluminal application intraoperatively to prevent vasospasm during the manipulations of the IMAs. The therapeutic concentration in plasma determined for isradipine ranges from 2–27 nM.19 This concentration was not effective in relaxing theIMA segment contracted with norepinephrine or the thromboxane A2 analogue (EC50 = 8 and 10 μM for norepinephrine- and the thromboxane A2 analogue-induced contractions, respectively).

The metabolism of nitroglycerin involves enzymatic and nonenzymatic pathways that generate nitric oxide.20 Nitric oxide produces vasodilation in activating soluble guanylate cyclase, and the consequent formation of cyclic guanosine monophosphate in the smooth muscle cell leads to the smooth muscle relaxation.17 Organic nitrate esters remain the most potent vasodilator in vitro22 and in vivo compared with other pharmacologic agents. Unfortunately, development of tolerance to nitrate may occur. The mechanisms include a reduction of nitrate biotransformation and neurohumoral reflex mechanisms, which are not yet completely understood.20 In the current experiments, all vessels relaxed to nitroglycerin, although 6 of the 12 patients were exposed to nitrate therapy at variable times before surgery.

Milrinone also completely reversed both norepinephrine- and the thromboxane A2 analogue-induced contraction. Thorin-Trescoes et al. demonstrated a greater effect of milrinone compared with sodium nitroprusside.13 Recently, Liu et al. showed that the vasodilator effect of milrinone is endothelium-independent.22 Milrinone is a derivative of bipyridine that selectively inhibits the phosphodiesterase type III and prevents the degradation of cyclic adenosine monophosphate. Its vasodilator effect on human IMA and the relation between the EC50 value and the therapeutic level are in agreement with previous studies.14 Papaverine, a benzylisoquinoline-derived and a nonspecific phosphodiesterase inhibitor, prevents the degradation of both cyclic adenosine monophosphate and cyclic guanosine monophosphate and is used exclusively in topical applications.

Prostaglandin E1 was ineffective at completely reversing thromboxane A2 analogue-induced vasoconstriction in IMA. Prostaglandin E1 activates adenyl cyclase independently of the β-receptors and increases production of cyclic adenosine monophosphate. The use of prostaglandin E1 has been suggested in association with norepinephrine in the treatment of pulmonary hypertension with right ventricular failure.23 Its rapid pulmonary metabolism, which may decrease its systemic vasodilator effect, is another potential advantage of prostaglandin E1 in this indication. Prostaglandin E1, however, like prostaglandin I2 (prostacyclin), has a profound inhibitory effect on platelets caused by stimulation of cyclic adenosine monophosphate.24 Further, in a clinical trial, a synergistic action of prostaglandin E1

Table 3. Effects of Different Vasodilator Agents in IMAs Precontracted with the Thromboxane A2 Analog

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>EC50 (nM)</th>
<th>P Value</th>
<th>Emax (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroglycerin</td>
<td>6</td>
<td>4.8 ± 6.8 x 10^-8</td>
<td>94 ± 2</td>
<td></td>
</tr>
<tr>
<td>Milrinone</td>
<td>7</td>
<td>4.9 ± 4.0 x 10^-9</td>
<td>&lt;0.01</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>Papaverine</td>
<td>5</td>
<td>4.4 ± 2.4 x 10^-8</td>
<td>&lt;0.01</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>Prostaglandin</td>
<td>6</td>
<td>6.8 ± 1.2 x 10^-7</td>
<td>&lt;0.01</td>
<td>73 ± 6</td>
</tr>
<tr>
<td>Isradipine</td>
<td>5</td>
<td>3.2 ± 2.4 x 10^-9</td>
<td>&lt;0.01</td>
<td>99 ± 1</td>
</tr>
</tbody>
</table>

Data are mean ± SD; n is the number of patients.

EC50 = the concentration that caused 50% relaxation of the thromboxane A2 analog-induced constriction; Emax = the maximal relaxation.

* The P value defines the difference between the EC50 of nitroglycerin and the EC50 of the other vasodilator drugs.

Anesthesiology, V 88, No 6, Jun 1998
Table 4. Mechanisms of Action of Different Vasodilator Agents

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug Class</th>
<th>Pathways</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroglycerin</td>
<td>Nitrovasodilator</td>
<td>Nitric oxide donor, stimulate guanylate cyclase to increase cGMP</td>
<td>Nitrate tolerance, venodilation</td>
</tr>
<tr>
<td>Milrinone</td>
<td>Bipyridine-PDE inhibitor</td>
<td>PDE type III inhibition, to inhibit breakdown cAMP</td>
<td>Positive inotropic effect, systemic vasodilation</td>
</tr>
<tr>
<td>Papaverine</td>
<td>Benzylinquinoine PDE inhibitor</td>
<td>PDE types I, II, and III inhibition to inhibit cAMP and cGMP breakdown</td>
<td>Platelet inhibition, systemic vasodilation</td>
</tr>
<tr>
<td>Prostaglandin E₁</td>
<td>Prostaglandins</td>
<td>Adenylyl cyclase activation to increase cAMP</td>
<td>Platelet inhibition, systemic vasodilation</td>
</tr>
<tr>
<td>Isradipine</td>
<td>Dihydropyridine Calcium channel blocker</td>
<td>L-type calcium channel inhibition to decrease calcium entry</td>
<td>Platelet inhibition, systemic vasodilation</td>
</tr>
</tbody>
</table>

PDE = phosphodiesterase; cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate.

and a nitric oxide donor in reducing platelet function has been established.²⁴

The dihydropyridine calcium channel inhibitor, isradipine, produced a vasodilator effect in IMA contracted with the thromboxane A₂ analogue. Vasodilation is achieved, however, at higher concentrations than the therapeutic concentrations in plasma reported in patients.¹⁹ Data regarding the effect of calcium channel inhibitors on precontracted IMA are variable. It appears that different calcium blockers, such as verapamil or the dihydropyridine agents nifedipine and nicardipine, showed a greater vasodilator effect when the vessel constriction was mediated by a voltage-dependent mechanism (i.e., KCl) rather than mediated by a receptor-dependent mechanism (i.e., the thromboxane A₂ analogue).¹¹,²⁵ Another perspective concerns the potential negative inotropic and conductive effects of nondihydropyridine agents (e.g., verapamil, diltiazem) related to the nonselective inhibition of L-type calcium channels located in the myocardium. Clevidipine, a third-generation ultra-short-acting dihydropyridine agent, however, appears to have a potent and selective vasodilator effect.²⁰⁺

Limitations

Although the segments of IMA are human tissue samples, the in vitro experiments exclude most of the mechanisms of the vascular tone regulation, such as the sympathetic reflexes or the responses to the shear stress. Moreover, as shown in the tables, there is a tremendous variability in vasodilator and vasoconstrictive effects among the vessel segments. The atherosclerotic process is likely involved in these changes. Consequently, the EC₅₀ value might not be correlated with the dose leading to an expected vasodilator effect in vitro. We also used different vasoconstrictor agonists separately because the IMA vasospasm mechanisms may be different to simultaneous neurohumoral stimulations, such as catecholamines, prostaglandins, vasopressin, and renin-angiotensin release.⁹

The current data demonstrate that the most commonly used vasodilator agents, except prostaglandin E₁, although they act through different pathways, effectively reverse precontracted IMAs in vitro at concentrations similar to those encountered clinically.

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Anesthesiology, V 88, No 6, Jun 1998
IN VITRO EFFECTS OF VASODILATOR AGENTS


