Nitrate tolerance: the lack of effect of N-acetylcysteine

JOHN O. PARKER, M.D., BERNICE FARRELL, R.N., KAREN A. LAHEY, AND BARRY F. ROSE, M.D.

ABSTRACT The hemodynamic and antianginal effects of 30 mg of isosorbide dinitrate (ISDN) were assessed in 12 patients with chronic stable angina after initial dosing and after 7 to 10 days of therapy four times daily. During early therapy, ISDN produced significant hemodynamic and antianginal effects that persisted over a 3 hr observation period. During sustained therapy there was attenuation of the hemodynamic effects at rest, and treadmill exercise time to the onset of angina and to the development of moderate angina was increased 1 hr after dosing; no effect was apparent at 3 hr. During this state of nitrate tolerance, patients were treated with an infusion of normal saline or 100 mg/kg N-acetylcysteine and exercise testing was repeated. N-Acetylcysteine did not change the hemodynamic findings at rest or during exercise and there was no improvement in exercise tolerance. It is apparent that the short-term administration of reduced sulphydryl groups does not reverse tolerance to the hemodynamic and antianginal effects of isosorbide dinitrate in an exercise test model.


IT HAS BEEN RECOGNIZED for many years that the administration of organic nitrates is associated with the rapid development of tolerance. This has been demonstrated in experimental animals, in which continued nitrate administration led to diminished hypotensive effects and increasing amounts of nitrates could be administered over short periods of time with greatly reduced hemodynamic effects. Tolerance has also been demonstrated in munitions workers and in patients treated with organic nitrates for angina pectoris and left ventricular failure.

The most widely accepted theory for the mechanism of tolerance is that during continued nitrate exposure there is a deficiency of reduced sulphydryl groups in vascular smooth muscle. This leads to diminished S-nitrosothiol and cyclic guanosine monophosphate production. The latter is considered responsible for nitrate-induced vasodilatation. Other explanations include plasma volume expansion and augmented neurohumoral responses with activation of the renin angiotensin system, changes that could modify the vasodilator effect of the nitrates.

This study was designed to test the hypothesis that deficiency of reduced sulphydryl groups during nitrate therapy is responsible for the development of tolerance and that the administration of sulphydryl groups will reverse tolerance.

Methods

Twelve patients with chronic, stable angina pectoris entered and completed this investigation. Coronary artery disease was documented by previous myocardial infarction or arteriographic evidence of significant coronary artery disease (>75% narrowing of at least one major coronary artery). Patients also experienced typical exertional angina and had positive treadmill tests as defined by the development of chest pain during exercise testing with the appearance of ischemic ST segment depression (horizontal or downsloping ST segment depression of at least 1 mm for 80 msec after the J point on the electrocardiogram). Patients were not included if they had unstable angina, myocardial infarction within 3 months, or congestive heart failure, or if they were taking digitalis glycosides, calcium antagonists, oral nitrates, or vasodilators other than sublingual nitroglycerin. One patient was receiving propranolol and this was maintained during the investigation but was not administered on study days until after the final treadmill test. Patients underwent preliminary exercise testing and the times to the onset of angina (P₁) and to the development of moderate angina (P₂) were documented. Moderate angina was defined as pain of such severity that patients would normally stop activity and take sublingual nitroglycerin. Patients were eligible for this investigation if the end point of moderate angina developed by the end of stage III of the Bruce protocol and if the end point was reproducible. Reproducibility was defined as the development of P₂ during two consecutive exercise tests within 60 sec of each other when exercise was stopped during stage I or II or within 90 sec when exercise was stopped during stage III of the Bruce protocol. Patients also had to show nitrate responsiveness, defined as an
increase in treadmill walking time of at least 60 sec when exercise was carried out 5 min after the sublingual administration of 0.4 mg of nitroglycerin. The study consisted of an early and a sustained phase.

**Early phase.** After fulfilling the entry criteria and when none of the exclusion criteria were present, patients entered the early phase of the investigation. In this study patients came to the laboratory in the morning in the fasting state without taking any morning medications. Hemodynamic observations were carried out at rest and the patients underwent treadmill testing. Time to the onset of angina (P₁) and to the development of moderate angina (P₂) was recorded. Patients then received in a random- ized, double-blind, crossover design, 30 mg of isosorbide dinitrate (ISDN) or matching placebo and treadmill testing was carried out after 1 and 3 hr. These early studies were separated by at least 2 days.

**Sustained phase.** After the early studies, patients were given ISDN, 30 mg qid (7 A.M., 12 P.M., 5 P.M., 11 P.M.), for 7 to 10 days. After this period of sustained therapy, patients came to the laboratory in the morning in the fasting state without taking their morning medications. Treadmill exercise testing was carried out, followed by administration of the morning dose of ISDN and exercise testing after 1 and 3 hr. In a randomized, double-blind fashion, patients then received an intravenous infusion, over a 15 min period, of 200 ml containing either normal saline or 100 mg/kg N-acetylcysteine, and exercise testing was carried out immediately thereafter. Ten minutes after this exercise test, the patients were given 0.4 mg of sublingual nitroglycerin and exercise testing was repeated after 5 min. The dosing schedule for ISDN was continued and the patient returned to the laboratory 2 days later, undergoing the same protocol and administration of the other infusion.

**Data analysis.** During the two early and sustained study days the significance of change at 1 and 3 hr from the corresponding control values was determined by paired t tests with the Bonferroni modification. A change was considered to be significant if a value of <.01 was found.

**Results**

All patients completed both phases of this investigation. During the infusion of N-acetylcysteine two patients developed mild reactions consisting of a sensation of flushing and pruritus, but the study protocol was completed despite these side effects.

**Early phase.** The hemodynamic and treadmill exercise testing data during early therapy with 30 mg of ISDN and placebo are shown in table 1 and figure 1. After oral ISDN, the resting standing systolic blood pressure fell significantly at 1 and 3 hr (p<.001) and resting heart rate increased (p<.001). Treadmill walking time to P₁ and P₂ was prolonged at 1 and 3 hr (p<.001). The exercise heart rate at P₂ at 1 and 3 hr was greater than that during the control exercise period (p<.01) but systolic blood pressure was similar. The rate pressure product at P₁ and P₂, 1 and 3 hr after administration of ISDN was similar to that during placebo therapy. When hemodynamic data for the 1 and 3 hr exercise tests were examined at the time of P₂ for the control study, the rate pressure product was lower at 1 hr (p<.01) but not at 3 hr. ST segment depression at P₂ was similar at each testing time for active and placebo therapy, whereas the time to 1 mm ST segment depression was significantly prolonged 1 and 3 hr after ISDN (p<.01) but not after placebo.

**Sustained phase.** The resting hemodynamic data and exercise testing results during sustained ISDN therapy are shown in table 2 and figure 2. Studies were carried out before and after the morning dose of ISDN on two occasions, and thus two sets of data are available before and 1 and 3 hr after the oral administration of ISDN. There were no significant differences between the values on these 2 days. Treadmill walking time to P₁ and P₂ was increased at 1 hr (p<.001) but not at 3 hr in comparison to control exercise values. The rate pressure product at P₂ was increased at 1 hr (p<.01) but not at 3 hr. When the rate pressure product for the 1 and

**TABLE 1**

<table>
<thead>
<tr>
<th>Hemodynamic and treadmill exercise data during early therapy (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise</strong></td>
</tr>
<tr>
<td>SBP</td>
</tr>
<tr>
<td>ISDN</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>1 hr</td>
</tr>
<tr>
<td>3 hr</td>
</tr>
<tr>
<td>Placebo</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>1 hr</td>
</tr>
<tr>
<td>3 hr</td>
</tr>
</tbody>
</table>

SBP = standing systolic blood pressure (mm Hg); HR = heart rate (beats/min); TWT = treadmill walking time (sec); RPP = rate pressure product (mm Hg/min·10⁻²); P₁ = onset of angina; P₂ = development of moderate angina.

Significance of change from control values: *p < .001; **p < .01.

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FIGURE 1. Treadmill walking time (TWT) to the development of moderate angina (P2) during early administration of 30 mg of ISDN and placebo. TWT was significantly increased at 1 and 3 hr over control and placebo values.

3 hr tests was calculated at the same time as P2 during the control test, there were no significant differences. Systolic blood pressure and heart rate at rest and during exercise were similar after the infusions of saline and N-acetylcysteine. Exercise time to P1 and P2 after the infusion of N-acetylcysteine was less than the control and 3 hr values (p<.01), and the time to P1 and P2 was not changed by the saline. The time to 1 mm ST segment depression was increased 1 hr after ISDN (p<.01) but not at 3 hr, but neither N-acetylcysteine nor normal saline altered the time to 1 mm ST depression.

Sublingual nitroglycerin decreased resting systolic blood pressure and increased heart rate and exercise time to P1 and P2 after both infusions (p<.001). When the effects of nitroglycerin were compared on the 2 infusion days, exercise time was greater when nitroglycerin was given after normal saline than after N-acetylcysteine (p<.01). The time to 1 mm ST depression was significantly prolonged by sublingual nitroglycerin (p<.001).

Discussion
This investigation has confirmed earlier studies demonstrating that tolerance to the hemodynamic and anti-

TABLE 2
Hemodynamic and treadmill exercise data during sustained ISDN therapy (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>P1</th>
<th>P2</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP</td>
<td>HR</td>
<td>TWT</td>
<td>SBP</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>121 ± 3.9</td>
<td>66 ± 3.6</td>
<td>148 ± 5.9</td>
<td>109 ± 4.7</td>
</tr>
<tr>
<td>1 hr</td>
<td>117 ± 3.6</td>
<td>74 ± 4.2B</td>
<td>148 ± 5.2</td>
<td>120 ± 5.7</td>
</tr>
<tr>
<td>3 hr</td>
<td>116 ± 3.0</td>
<td>74 ± 4.3B</td>
<td>144 ± 5.6</td>
<td>116 ± 4.8</td>
</tr>
<tr>
<td>NAC</td>
<td>125 ± 4.6</td>
<td>76 ± 3.6</td>
<td>152 ± 7.4</td>
<td>116 ± 4.6</td>
</tr>
<tr>
<td>GTN&lt;sub&gt;SL&lt;/sub&gt;</td>
<td>108 ± 5.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>91 ± 4.0&lt;sup&gt;A&lt;/sup&gt;</td>
<td>156 ± 8.2</td>
<td>127 ± 6.1B</td>
</tr>
</tbody>
</table>

Day 2

|          |           |            |            |          |          |           |           |                |
|----------|-----------|------------|------------|          |          |           |           |                |
| Control  | 129 ± 5.3 | 69 ± 3.7   | 152 ± 6.9  | 110 ± 5.1 | 241 ± 26.8 | 158 ± 9.1 | 119 ± 6.6 | 191 ± 19.4 | 335 ± 25.9 | 272 ± 30.3<sup>a</sup> |
| 1 hr     | 119 ± 5.8 | 73 ± 3.5   | 152 ± 7.4  | 120 ± 5.0 | 354 ± 29.1<sup>a</sup> | 159 ± 8.2 | 129 ± 5.5 | 209 ± 17.4 | 436 ± 26.6 | 376 ± 35.6<sup>a</sup> |
| 3 hr     | 122 ± 6.1 | 76 ± 3.9   | 144 ± 7.1  | 115 ± 4.6 | 274 ± 27.2 | 153 ± 8.5 | 127 ± 6.1 | 194 ± 15.4 | 364 ± 32.0 | 308 ± 36.1       |
| Saline   | 125 ± 5.5 | 77 ± 3.3   | 150 ± 8.4  | 121 ± 6.1 | 283 ± 32.0 | 160 ± 9.7 | 130 ± 6.6 | 211 ± 19.7 | 374 ± 32.0 | 288 ± 35.1       |
| GTN<sub>SL</sub> | 110 ± 4.8<sup>a</sup> | 87 ± 4.0<sup>a</sup> | 148 ± 10.0 | 128 ± 7.4B | 376 ± 41.5A | 152 ± 9.7 | 135 ± 6.4<sup>a</sup> | 209 ± 18.3 | 445 ± 29.4<sup>b</sup> | 378 ± 43.9<sup>a</sup> |

NAC = N-acetylcysteine; GTN<sub>SL</sub> = sublingual nitroglycerin; other abbreviations as in table 1.
Significance of change from control values: <sup>A</sup>p < .001; <sup>B</sup>p < .01. For data after nitroglycerin, the significance of change is from previous values (i.e., after either NAC or saline).
anginal effects of the organic nitrates develops rapidly during therapy four times daily. Thus, during early therapy with 30 mg of oral ISDN, there was a decrease in resting systolic blood pressure and an increase in heart rate at 1 and 3 hr after dosing, and exercise duration to P1 and P2 was significantly prolonged at both of these testing times. When therapy four times daily was continued for 7 to 10 days, the resting hemodynamic effects were significantly attenuated and treadmill walking time was increased at 1 hr but not 3 hr after the morning dose. Although it now is generally recognized that tolerance does occur during sustained therapy, earlier studies have reported that antianginal efficacy is present during sustained ISDN therapy.16, 17

The mechanism of action of the organic nitrates is not completely understood. Needlemann and Johnson18 concluded that the relaxation by nitroglycerin of precontracted aortic strips was dependent on the presence of reduced sulphydryl groups in these tissues. More recent observations by Ignarro et al.13 have demonstrated that nitrogen oxide-containing vasodilator drugs, i.e., organic nitrates and nitrates, sodium nitrate, and nitroprusside, react with reduced sulphydryl groups leading to the production of S-nitrosothiols. The latter compound stimulates guanylate cyclase with resultant elevation of cyclic guanosine monophosphate, which induces vasodilatation either by reducing calcium uptake into cells or increasing calcium uptake by sarcoplasmic reticulum.

Tolerance to the nitrates has been considered to be secondary to depletion of reduced sulphydryl groups in vascular smooth muscle,13, 18 and Needlemann and Johnson18 also demonstrated that disulfide-reducing agents can reverse nitrate tolerance. Thus the addition of dithiothreitol restored responsiveness in rabbit aorta strips made tolerant to nitroglycerin. Studies in vivo were carried out in rats made tolerant by repeated large subcutaneous injections of nitroglycerin. The aortic strips from these tolerant rats showed vasodilator effects only with large doses of nitroglycerin. Dithiothreitol added to these preparations was effective in restoring responsiveness to nitroglycerin. Other studies by Needlemann et al.12 demonstrated that ethacrynic acid, which causes alkylation of tissue thiol groups, induced refractoriness to the vasodilating effect of nitroglycerin in aortic strips.

Studies have suggested that the administration of reduced sulphydryl groups potentiated the effect of nitroglycerin. In a study carried out in man, the effects of nitroglycerin on mean arterial blood pressure and pulmonary capillary wedge pressure were recorded.19 N-Acetylcysteine was then infused and the dose of nitroglycerin required to lower systemic arterial pressure and left ventricular filling pressures was significantly decreased. The authors concluded that N-acetylcysteine potentiated the vasodilator effects of nitroglycerin. In a more recent investigation, Winniford et al.20 studied the effect of nitroglycerin on coronary sinus blood flow in patients with coronary sinus blood flow induced by nitroglycerin. These studies suggested that N-acetylcysteine, a source of reduced sulphydryl groups, potentiated the vasodilating effects of nitroglycerin. Both of these investigations used the same dosing schedule as the present study.

Torresi et al.21 studied the effect of N-acetylcysteine in the prevention of nitrate tolerance in incubated bovine coronary artery rings. The degree of tolerance that developed during nitrate administration was markedly reduced by administration of this sulphydryl group donor. Few data are available to assess nitrate tolerance in man. In a recent publication, Packer et al.22 studied patients with congestive heart failure. Infusion of nitroglycerin reduced systemic pressure and left ventricular filling pressures. As the infusion continued throughout a 48 hr period, the hemodynamic variables returned to control values, indicating the development of tolerance. In a subset of seven patients, 200 mg/kg N-acetylcysteine was administered orally, resulting in the restoration of hemodynamic values to those seen during the initial period of nitroglycerin infusion.

There have been no reports of the effects of N-acetylcysteine on exercise performance in patients made tolerant to the organic nitrates. The short-term administration of reduced sulphydryl groups does not appear to reverse the tolerance to the hemodynamic and antianginal effects of ISDN in an exercise test model. There were no changes in the hemodynamics at rest or during exercise and more importantly exercise duration to the onset of angina and to the development of moderate angina was not increased when N-acetylcysteine was given 3 hr after a dose of isosorbide dinitrate, when nitrate tolerance was clearly documented. It has been suggested the N-acetylcysteine augments the vasodilator response of the organic nitrates20 but our study does not support these observations. Thus when sublingual nitroglycerin was administered, the decrease in systolic blood pressure was similar in patients given N-acetylcysteine and saline. Likewise, the improvement of exercise performance after nitroglycerin was not increased by N-acetylcysteine and was actually less than that after the infusion of saline. It is possible that the dose of N-acetylcysteine was too small or that the kinetics of this agent are such that insufficient time was permitted for distribution to vascular smooth
muscle. Little is known of the pharmacokinetics of N-acetylcysteine, but the timing of observations was similar to those carried out by Horowitz et al. and Winniford et al. The study of Packer et al., in which N-acetylcysteine was given orally, did provide longer time frame and suggested reversal of nitrate tolerance. Thus it is possible that our studies were carried out too soon after administration of N-acetylcysteine. The study did not address the issue whether this sulphydryl group donor would prevent tolerance if administered early during therapy.

Other possibilities for nitrate tolerance include the development of an increased plasma volume, which would diminish the hemodynamic effect of nitrate-induced vasodilation. Other suggestions to explain nitrate tolerance include activation of neurohumoral mechanisms. Thus it has been suggested that activation of the renin angiotensin system by vasodilators may attenuate the hemodynamic and antianginal effects of the organic nitrates. These hypotheses have not been assessed as yet in patients with chronic stable angina pectoris.

The present investigation has not addressed the question of nitrate tolerance in patients treated for left ventricular failure. Although tolerance to the organic nitrates has been demonstrated in heart failure, the mechanisms may be different from those that occur during therapy for angina pectoris.

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