

Preventive Administration of Intravenous N-Acetylcysteine and Development of Tolerance to Isosorbide Dinitrate in Patients With Angina Pectoris

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Background. Development of tolerance to organic nitrates may be related to depletion of sulfhydryl groups in vascular smooth muscle. N-Acetylcysteine (NAC), a sulfhydryl donor, has been reported to potentiate the effect of nitroglycerin and reverse tolerance in humans. However, its ability to prevent or delay the development of nitrate tolerance in patients with angina pectoris has not been established.

Methods and Results. Ten patients with stable angina pectoris were treated with intravenous isosorbide dinitrate (ISDN; 5 mg/hr) combined with NAC (2 g i.v. over 15 minutes followed by 5 mg/kg/hr) or matching placebo for 30 hours in a double-blind, randomized, crossover study with a washout interval of 8 days. Bicycle exercise tests were performed before and at 1½, 8, 20, 24, and 30 hours after start of treatment. After 24 hours of infusion, exercise parameters were not significantly different from pretreatment values ($p > 0.05$) during ISDN plus placebo, indicating development of tolerance to ISDN. In contrast, time to onset of angina, time to 1-mm ST segment depression, and total amount of ST segment depression were still significantly improved after 24-hour infusion of ISDN plus NAC ($p < 0.05$). In addition, compared with placebo, a significant difference ($p < 0.05$) in favor of NAC was observed regarding time to angina (507 ± 63 versus 445 ± 69 seconds, mean \pm SEM), time to 1-mm ST segment depression (435 ± 43 versus 407 ± 45 seconds), and total ST segment depression (1.8 ± 0.9 versus 3.1 ± 0.4 mm).

Conclusions. These results suggest that infusion of high doses of NAC in combination with ISDN for 30 hours affects and partially prevents the development of tolerance to antianginal effects normally observed during infusion with ISDN. (*Circulation* 1992;85:143-149)

The magnitude and duration of the acute circulatory and antianginal effects of organic nitrates are reduced during sustained treatment.¹⁻³ Furthermore, tolerance to isosorbide dinitrate (ISDN) and nitroglycerin (NTG) may develop within 18-24 hours after initiation of therapy.⁴⁻⁷ An important mechanism by which this nitrate tolerance occurs appears to involve depletion of sulfhydryl groups, which are essential for biotransformation of nitrates to vasoactive S-nitrosothiols and nitric oxide.⁸⁻¹⁰ Also, neurohormonal counterregulatory mechanisms and plasma volume expansion may contribute to the development of tolerance.^{6,11,12}

Recent studies have shown that sulfhydryl donors potentiate the acute effect of NTG.^{13,14} In contrast, results regarding reversal of tolerance are more controversial, showing reversal in some studies^{6,7,15} and not in others.¹⁶⁻¹⁸ Clinical data about the use of sulfhydryls in prevention of tolerance are limited,¹² and it is presently unknown whether administration of sulfhydryl groups prevents or delays the development of tolerance to the antianginal effects of ISDN in patients with angina pectoris.

Therefore, the present study was performed to assess whether simultaneous infusion of ISDN and the sulfhydryl donor N-acetylcysteine (NAC) prevents tolerance development to ISDN in patients with stable angina pectoris.

Methods

Patients

Study inclusion criteria were coronary artery disease confirmed by coronary angiography, exertional angina pectoris reproducible at exercise testing (de-

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defined as variability of exercise time to onset of angina of less than 1 minute on consecutive tests) and associated with at least 0.1-mV ST segment depression measured 80 msec after the J point. An increase in exercise tolerance to onset of angina by at least 30 seconds after sublingual NTG, despite the use of other antianginal medications, was necessary as evidence of initial nitrate responsiveness.

Bicycle exercise tests were performed in 12 male patients with stable angina pectoris. All were nitrate responders, but in two patients exercise results could not be reproduced. Thus, 10 patients (mean age, 55 years; range, 44–75 years) were included in the study. One patient had one-vessel, four had two-vessel, and five had three-vessel disease. Of the 10 patients, one patient did not use antianginal medication, eight patients received a calcium antagonist (verapamil, seven; nifedipine, one), and one patient received a β -adrenergic blocker (metoprolol). This treatment was continued unchanged throughout the study period. None of the patients received digoxin, and long-acting nitrate therapy was discontinued 1 week before the study. Sublingual NTG was allowed to relieve acute attacks of angina pectoris, except for 1 hour before the start of each study period. Angina pectoris had been present from 6 months to 3 years, and five patients had a documented myocardial infarction occurring more than 3 months before the study period. None of the patients showed clinical signs of pulmonary disease, left ventricular failure (mean ejection fraction, 52%; range, 36–64%), arterial hypertension, valvular heart disease, conduction disturbances, renal disease, or other conditions that may limit exercise testing.

Informed consent was obtained from all patients, and the study was approved by the Ethical Committee of Copenhagen.

Protocol Design

Two preliminary exercise tests were performed between 9:00 AM and 11:00 AM on two different days to verify exercise tolerance reproducibility.

The investigation was performed as a double-blind, randomized, crossover trial. Each of the two study periods lasted for 30 hours, and they were separated by a washout interval of 1 week. During both study periods, patients received a continuous infusion of ISDN combined with either NAC or matching placebo. ISDN (5 mg/hr [20 ml/hr]; Schwarz, Germany) was infused in polyethylene tubes in a solution of 5% glucose for 30 hours with an automatic infusion pump (Abbott Laboratories, Chicago). NAC (ASTRA AB, Sweden) was infused at a rate of 2 g in 100 ml saline over 15 minutes followed by an infusion dose of 5 mg/kg/hr (12–16 ml/hr adjusted according to weight) for 30 hours. (ASTRA AB, Sweden [NAC, Mucomyt] and Ercopharm A/S, Denmark [ISDN, Cardopax], generously supplied the drugs used). Even higher boluses of NAC have not been reported to affect hemodynamic parameters in patients with angina pectoris.^{7,13,14} Comparable high doses of NAC

are used safely in the treatment of acetaminophen overdose.

During both study periods, exercise testing, blood pressure measurements, and heart rate measurements were carried out before the start of infusions (0 hour, 11:00 AM) and 1½, 8, 20, 24, and 30 hours after the onset of treatment. Measurements obtained immediately before the onset of infusion (0 hour) in each period were used as baseline values.

Exercise Protocol

Exercise tests were performed with a bicycle ergometer, beginning at a 25-W load with the work load progressively increased by 25 W every second minute to a symptom-limited end point consisting of anginal pain that would stop daily life activities (total exercise time). Systolic blood pressure was recorded before exercise; at the end of each work load; and 1, 2, 4, and 10 minutes after exercise. A six-precordial-lead ECG was recorded continuously for measurement of heart rate and surveillance of ST segment changes. The amount of exercise-induced ST segment depression was used as the major objective parameter in determining exercise-induced myocardial ischemia. However, also analyzed from the exercise test were the variables of total exercise time, time to onset of angina, time to 1-mm ST depression, and the product of heart rate and systolic blood pressure (rate/pressure product) at maximal work loads. Time to 1-mm ST depression was studied in the lead that produced the largest depression during baseline exercise testing. This lead was used for comparison during the study. Total ST segment depression was calculated as the cumulative amount of ST depression in the six precordial leads at maximal comparable, intraindividually identical work loads.

Statistics

Results were analyzed using analysis of variance (ANOVA) to take into account three factors: treatment, infusion time, and treatment sequence. Comparison of variables on each study day was performed by one-way ANOVA. At each time point, differences between NAC and placebo were assessed by means of paired Student's *t* tests. Within each study period, changes between baseline values and values recorded 1½ and 24 hours after the start of infusion were considered relevant and useful for comparisons. Interpretation of differences between baseline values and values recorded at other time points (8, 20, and 30 hours) is difficult due to the influence of circadian variation on ischemic threshold and usual antianginal therapy (given several times a day). The intraindividual changes in each infusion period were calculated by subtracting the baseline from that obtained during infusion. One patient did not develop angina during treatment with ISDN plus or without NAC and was assigned the total exercise time in the statistical analysis regarding this parameter. Values are expressed as mean \pm SEM and 95% confidence intervals. For all analyses, a two-tailed *p* value of less than

TABLE 1. Effects of Intravenous Isosorbide Dinitrate Plus Placebo and Isosorbide Dinitrate Plus N-Acetylcysteine on Heart Rate, Blood Pressure, and Rate/Pressure Product at Maximal Exercise

		Time after treatment start (hours)					
		0	1.5	8	20	24	30
Heart rate (beats per minute)							
Supine	P	58±2	55±3	65±4	57±3	59±3	60±3
	N	58±2	56±2	66±4	68±3	64±3	65±4
Sitting	P	65±4	81±5*	80±6*	73±5*	69±5	74±5*
	N	67±2	78±6*	81±6*	80±4*	73±4	71±2*
Maximal	P	112±5	118±5	118±5	111±7	108±5	112±7
	N	109±5	120±7	120±8	113±8	112±7	114±7
Systolic blood pressure (mm Hg)							
Supine	P	143±6	119±3*	121±5*	119±6*	123±5*	127±4*
	N	138±8	116±5*	121±6*	118±5*	123±5*	123±4*
Sitting	P	135±6	109±5*	120±6*	106±6*	110±4*	119±4*
	N	130±5	109±4*	111±6*	113±4*	114±5*	120±5*
Maximal	P	171±6	160±8	158±8	150±9*	150±8*	154±8
	N	168±6	161±8	156±9	158±7	156±6*	160±7
Diastolic blood pressure (mm Hg)							
Supine	P	88±4	78±3	78±3	79±4	78±5	78±4
	N	86±4	78±4	78±4	78±3	78±4	81±3
Sitting	P	84±2	76±3*	74±3*	73±3*	78±4*	73±2*
	N	86±3	76±3*	73±3*	73±3*	78±4*	78±2*
Rate/pressure product (mm Hg/min×10 ⁻²)							
Maximal	P	192±14	195±17	188±16	164±17*	168±13	175±18
	N	181±15	195±18	191±21	179±23	179±16	182±20

P, placebo; N, N-acetylcysteine.

n=8.

*p<0.05 compared with pretreatment (0 hours).

0.05 was considered significant. *p* values were corrected using Bonferroni's adjustment.

Results

Ten patients were entered into the protocol; two subjects were withdrawn from the study. One patient experienced accelerated angina pectoris during infusion of ISDN (plus placebo), and one patient developed unstable angina pectoris in the interval between the two study periods. Headache was experienced in seven of the eight ISDN-plus-NAC infusion periods and in six of the eight ISDN-plus-placebo periods. In no case was it necessary to discontinue treatment because of this undesired effect.

Results are presented for the eight patients who completed the entire study. Preinfusion (baseline) hemodynamic data and exercise results were comparable on the two study days and did not differ significantly (Tables 1 and 2). No carryover effect was observed.

Resting Hemodynamics

The changes in hemodynamic values over the 30-hour study periods are shown in Table 1. Supine systolic blood pressure was acutely lowered during both infusion regimens and remained significantly decreased compared with baseline values throughout the study periods without any significant treatment difference. An increase in supine heart rate and a decrease in

supine diastolic blood pressure during infusions did not reach statistical significance. With patients in the upright position, these changes were augmented; heart rate was significantly increased, and systolic and diastolic blood pressures were significantly reduced compared with pretreatment values. The changes were maintained throughout the 30-hour infusions and were not affected by NAC administration.

Exercise Tolerance

Table 2 shows the results regarding exercise end points. Time to 1-mm ST segment depression and time to onset of angina were prolonged and significantly different after 8, 24, and 30 hours of infusion of ISDN plus NAC when compared with ISDN plus placebo (Table 2). Furthermore, the total amount of ST segment depression was reduced and significantly different from that of ISDN plus placebo after 20, 24, and 30 hours of treatment with ISDN plus NAC (Table 2).

Compared with baseline values, increases in time to 1-mm ST segment depression and time to angina were observed only after 1½ hours during infusion of ISDN plus placebo, whereas a significant increase was still present after 24 hours of infusion with ISDN plus NAC (Table 2 and Figures 1, 2, and 4). Compared with baseline, ST segment depression was reduced after 1½ hours during both infusion periods

TABLE 2. Effects of Isorbide Dinitrate Plus Placebo and Isorbide Dinitrate Plus N-Acetylcysteine on Time to Onset of Angina Pectoris, Time to 1-mm ST Segment Depression, Total ST Segment Depression, and Total Exercise Time

		Time after start of treatment (hours)					
		0	1.5	8	20	24	30
Time to onset of angina (seconds)	P	437±61	499±70*	457±60	442±61	445±60	418±57
	N	430±51	548±58*	510±57*†	460±53	507±63*†	459±56†
	Δ (N-P)	-7 (-68 to 54)	51 (-56 to 138)	53 (0.2 to 106)	18 (-31 to 97)	62 (5 to 119)	41 (1 to 81)
Time to 1-mm ST depression (seconds)	P	391±51	475±61*	428±44*	405±52	407±45	345±48
	N	353±49	493±57*	458±50*†	398±51	435±43*†	398±51†
	Δ (N-P)	-38 (-86 to 8)	18 (-89 to 124)	30 (3 to 57)	-7 (-38 to 24)	28 (3 to 53)	53 (3 to 102)
Total ST segment depression (mm)	P	3.1±0.6	1.5±0.6*	2.8±0.8	3.1±0.5	3.1±0.4	4.4±0.6
	N	3.1±0.5	0.5±0.3*	1.8±0.4*	2.4±1.1*†	1.8±0.9*†	3.1±0.5†
	Δ (N-P)	0 (-1.2 to 1.2)	-1 (-2.6 to 0.6)	-1 (-2.3 to 0.3)	-0.7 (-1.1 to -0.3)	-1.3 (-1.7 to -0.9)	-1.3 (-2.7 to 0.1)
Exercise time (seconds)	P	515±49	571±48*	531±41	508±52	513±45	485±45
	N	503±52	583±54*	559±51*†	505±50	545±65	492±52
	Δ (N-P)	-12 (-55 to 31)	12 (-21 to 45)	28 (5 to 52)	-3 (-51 to 46)	32 (-32 to 97)	7 (-40 to 56)

n=8.

*p<0.05 compared with pretreatment (0 hours).

†p<0.05 compared with placebo.

Values are mean±SEM, with 95% confidence intervals in parentheses.

and still reduced after 24 hours during ISDN plus NAC (Table 2 and Figures 3 and 4).

The increases (actual value minus baseline value) in time to 1-mm ST segment depression and time to angina were significantly different at 8, 24, and 30 hours during infusion of ISDN plus NAC compared with infusion of ISDN plus placebo (Figures 1 and 2).

Compared with ISDN plus placebo, the improvement in ST segment depression was significantly more pronounced at 20, 24, and 30 hours during ISDN plus NAC (Figure 3).

Total exercise time was increased after 1.5 hours but was not statistically different from baseline values after 24 hours in any of the treatment groups. A

increase in time to angina

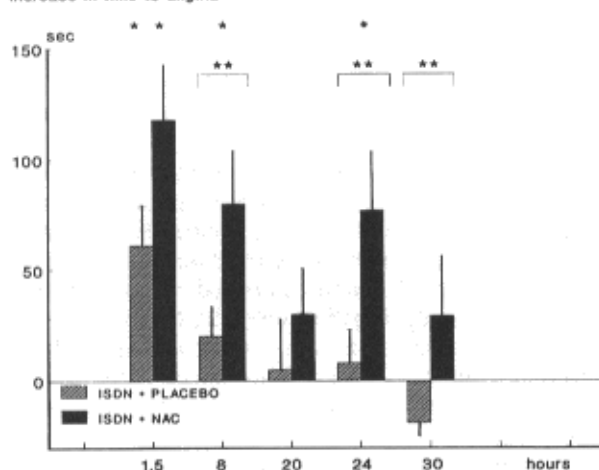


FIGURE 1. Bar graph of mean±SEM change from baseline value (0 hours) in time to onset of angina pectoris during intravenous infusion of isorbide dinitrate (ISDN) plus or without N-acetylcysteine (NAC) for 30 hours in eight patients with stable angina. 0, Time to angina observed before start of treatment. *p<0.05 compared with baseline. **p<0.05 compared with placebo.

increase in time to 1 mm ST depression

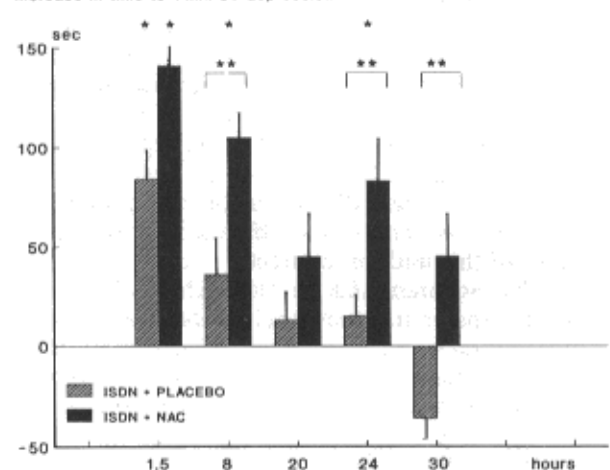


FIGURE 2. Bar graph of mean±SEM change from baseline value (0 hours) in time to 1-mm ST segment depression during intravenous infusion of isorbide dinitrate (ISDN) plus or without N-acetylcysteine (NAC) for 30 hours in eight patients with stable angina. 0, Time to 1-mm ST segment depression observed before start of treatment. *p<0.05 compared with baseline. **p<0.05 compared with placebo.

trend in favor of NAC reached statistical significance only at 8 hours (Table 2).

At peak exercise, alterations in the rate/pressure product were not statistically significant. There was no indication of a treatment difference regarding maximal heart rate and systolic blood pressure (Table 1).

Discussion

Several studies have shown that the magnitude and duration of the circulatory and antianginal effects of organic nitrates are reduced during sustained therapy.^{1,2,5,7} In the present study, an initial improvement in exercise parameters after 1½ hours of ISDN plus placebo infusion gradually declined; at high steady-state plasma nitrate concentrations (data not shown), exercise parameters after 24 hours were similar to preinfusion values. Veins rather than arteries may be the primary site of tolerance development,¹⁹⁻²¹ and as previously described by Zimrin et al,⁵ tolerance to the antianginal effects developed, although the nitrate effect on systemic blood pressure was still present. This is also in accordance with other findings showing that tolerance on right atrial pressure and pulmonary vascular resistance may develop more rapidly than tolerance on systemic vascular resistance and cardiac index.²²

Organic nitrates interact with thiols to generate labile vasoactive S-nitrosothiols and nitric oxide, which activates guanylate cyclase and causes smooth muscle relaxation.^{8,9} Because thiols are required for the metabolism of organic nitrates, the mechanism of tolerance development has been attributed to a depletion of sulfhydryl groups caused by prolonged exposure to nitrates.^{9,10,23,24} In line with this hypothesis, administration of the sulfhydryl donor NAC has produced reversal of tolerance in some *in vitro*^{15,23} and clinical studies.^{6,7} However, other studies have

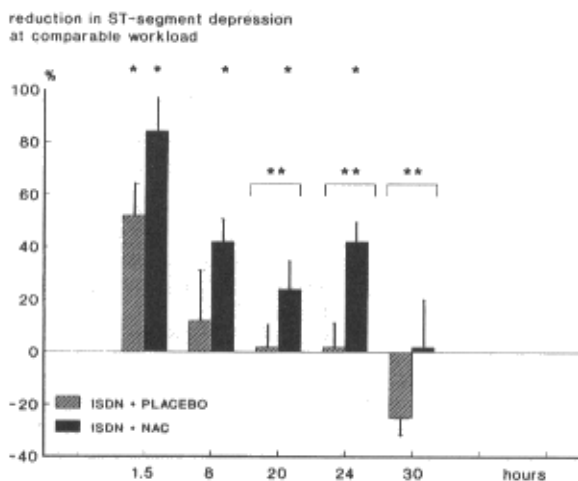


FIGURE 3. Bar graph of mean \pm SEM reduction in total ST segment depression at comparable work load during infusion of isosorbide dinitrate (ISDN) plus or without N-acetylcysteine (NAC). 0, ST segment depression observed before start of infusion. * $p < 0.05$ compared with baseline (0 hours). ** $p < 0.05$ compared with placebo.

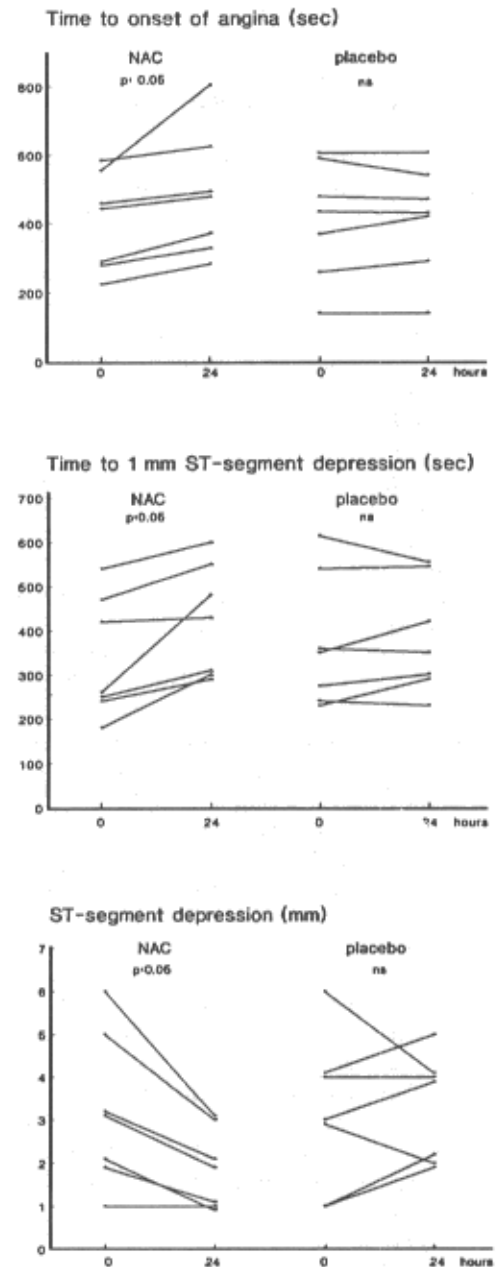


FIGURE 4. Plots of individual exercise data for eight patients with angina pectoris. 0 Hours, baseline values; 24 hours, values obtained after constant infusion of either isosorbide dinitrate (ISDN) plus placebo or ISDN plus N-acetylcysteine (NAC).

not been able to confirm these findings,¹⁶⁻¹⁸ and in general, results regarding reversal of tolerance by sulfhydryl supplementation are conflicting. More uniform experimental results have been reported regarding an effect of sulfhydryl donors in the prevention of tolerance development.^{15,25-27}

The present study addresses the question whether NAC in the clinical setting prevents the development of tolerance to ISDN. The results show that preventive NAC treatment modifies the development of tolerance to the antianginal effects of intravenous

ISDN in patients with ischemic heart disease. Compared with infusion of ISDN alone, time to onset of angina, time to 1-mm ST segment depression, and total ST segment depression were all improved when NAC was administered simultaneously with ISDN. Although Dupuis et al¹² concluded that the effect of NAC in prevention of tolerance in patients with congestive heart failure was negligible, they found that NAC prevented development of tolerance to the initial effect of nitroglycerin on mean right atrial pressure and partially prevented development of tolerance on pulmonary artery and wedge pressures.¹² These findings were recently emphasized by Packer,²⁸ and other studies also suggest that NAC may modify tolerance primarily in the venous vascular bed.^{20,29}

In the only previous study using ISDN, Parker et al¹⁶ reported that a bolus dose of NAC was unable to reverse existing nitrate tolerance in patients with stable angina pectoris. However, it is possible that sulfhydryls may be more likely to prevent tolerance than to abolish already existing tolerance,^{25,26} and results from the present study may therefore not necessarily contrast with the results by Parker et al.¹⁶

An unspecific extracellular thiol/NTG interaction and production of vasoactive nitrosothiols may potentiate the response to NTG regardless of whether a tolerant state exists.³⁰ Compared with NTG, ISDN appears not to be involved in a similar tolerance-independent nitrate/thiol interaction.³⁰ In agreement with this observation, the trend toward a more pronounced response to ISDN plus NAC before the onset of tolerance (1.5 hours) compared with ISDN plus placebo did not reach statistical significance. However, the possibility that a larger study population would have shown a real, significant difference has to be considered. The same reservation may be made regarding total exercise time, which was significantly prolonged by NAC only at 8 hours although other clinical (time to angina) and objective signs (ECG changes) of ischemia were still improved at later test periods. Alternatively, this finding may be due to a temporal dissociation between nitrate effects at maximal (total exercise time) and submaximal exercise (time to angina and ECG changes). A similar response was described by Zimrin et al,⁵ who found that exercise enhancement in patients with angina pectoris was abolished at a time when submaximal exercise effects of NTG persisted. Unfortunately, available data do not allow insight into the circulatory mechanisms responsible for this discrepancy.

NAC appeared to prevent antianginal tolerance development, but this effect was only partial. Despite continuous infusion of ISDN plus NAC, the initial changes in exercise parameters gradually decreased. Assuming continued availability of the sulfhydryl donor throughout the study period, the present data therefore also support an obligatory role for mechanisms for tolerance development other than sulfhydryl depletion. This is consistent with data suggesting that multiple mechanisms (e.g., tolerance to S-nitro-

sothiols, neurohormonal activation, and plasma volume expansion) interact and may contribute to the development of tolerance.^{6,12,31}

In the present study, we used a high dose of NAC. It is not known whether lower intravenous doses of NAC are similarly effective. However, in the general management of chronic stable angina, the use of smaller oral doses would be necessary because a high frequency of gastrointestinal side effects occurs with chronic administration.³² In a recent study with oral ISDN and NAC (600 mg q.i.d.), we found a small beneficial effect of NAC on ST segment depression during ISDN therapy, although the changes were less pronounced than in the present study.³³ It may therefore be possible that NAC is effective in the prevention of tolerance in relatively low doses compared with the high doses needed in the reversal of tolerance. Finally, NAC has free radical-scavenging properties³⁴ and may potentiate the effect of nitrates on platelet aggregation.³⁵ At present, however, additional studies are required to delineate the potential benefits of an interaction between different organic nitrates and different types and doses of sulfhydryl donor substances in the management of ischemic heart disease.

In conclusion, the present study represents the first reported clinical investigation into the prevention of nitrate tolerance by high intravenous doses of NAC in patients with angina pectoris. The results suggest that an infusion of NAC in combination with ISDN for 30 hours affects and partially prevents the development of tolerance to the antianginal effects normally seen during infusion with ISDN.

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