Different Susceptibility to the Development of Nitroglycerin Tolerance in the Arterial and Venous Circulation in Humans

Effects of N-Acetylcysteine Administration

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Background. Tolerance to the effects of organic nitrates develops rapidly during continuous exposure to these drugs; its main mechanism seems to be an intracellular sulfhydryl group depletion. However, the relative susceptibility to the development of nitroglycerin tolerance of the arterial or venous circulation in humans is still a matter of dispute.

Methods and Results. Twenty patients with coronary artery disease underwent a continuous 24-hour nitroglycerin infusion followed by a bolus administration of N-acetylcysteine. Forearm blood flow (ml/100 ml/min) and venous volume (ml/100 ml) were measured by strain gauge plethysmography under control conditions, at the end of nitroglycerin titration, after 24-hour infusion, and after N-acetylcysteine; vascular resistance was calculated as mean cuff blood pressure divided by flow. After 24 hours of nitroglycerin infusion, the initial increase in venous volume was reduced 48% (p<0.01), whereas the acute effects on vascular resistance were not attenuated in the whole group. N-Acetylcysteine completely restored nitroglycerin venodilator effects in all 10 patients in whom attenuation of the venous effects was observed during the infusion period.

Conclusions. The data indicate that the susceptibility to the development of nitrate tolerance in humans is higher in the venous than in the arterial circulation, and that the sulfhydryl group donor N-acetylcysteine is extremely effective in reversing nitroglycerin tolerance in the venous circulation in humans. (Circulation 1992;86:798–802)

Key Words • nitrate tolerance • N-acetylcysteine • plethysmography

Many clinical studies, both of patients with angina pectoris and of patients with heart failure,5–7 have demonstrated that continuous administration of organic nitrates leads to a decrease of their therapeutic efficacy. Nevertheless, there is still no general agreement concerning the relative susceptibility of the arterial and venous system to the development of nitrate tolerance in humans. In 1975, Zelis and Mason8 demonstrated venous but not arterial cross-tolerance to the effects of isosorbide dinitrate and nitroglycerin in six normal volunteers. Subsequent studies, however, reported different results, either showing partial tolerance in both arterial and venous circulations9 or demonstrating significant attenuation to the arterial and not to the venous or pulmonary vascular effects of isosorbide dinitrate.10 More recently, the greater and more frequent attenuation of nitroglycerin action in the venous bed has been reemphasized in nine patients with congestive heart failure.11

Accordingly, the aim of our study was to assess the specific responses to a 24-hour continuous nitroglycerin infusion of the peripheral arterial and venous vascular circulations in humans. In addition, because intracellular sulfhydryl groups are necessary for nitrate-induced vasodilation12,13 and a reduction in tissue content of these groups is thought to be one of the primary mechanisms responsible for nitrate tolerance,14,15 we also investigated the effects of N-acetylcysteine, a sulfhydryl-containing compound, on the arterial and venous tolerant and nontolerant circulations.

Methods

Patients

Twenty patients with coronary artery disease entered and completed the investigation (18 men, two women; mean age, 56 years). Coronary artery disease was documented by coronary arteriography or considered to be present in case of medical history of previous Q wave myocardial infarction. Antianginal medications (β-blocking agents, calcium antagonists, nitrates) were discontinued 48–72 hours before the study; chronic therapy with diuretic drugs was not modified. Patients with unstable angina, heart failure, hypertension (diastolic pressure >95 mm Hg), hypotension (systolic pressure <100 mm Hg), or atrial fibrillation were not included. Each patient was informed, and consent was obtained before the study.


Hemodynamic Measurements

Any hemodynamic determination was performed with patients in supine position, in a quiet, warm environment for at least 30 minutes. Forearm blood flow (ml/100 ml/min) was measured by the venous occlusion technique with a mercury-in-silastic strain gauge plethysmograph as previously described. The strain gauge was placed approximately 5 cm below the antecubital crease with the arm supported above the heart level to ensure that forearm venous pressure was less than 1 mm Hg before volume measurements; a venous occlusion cuff was placed at the upper arm (inflation pressure was 40 mm Hg). Forearm blood flow was calculated from the rate of increase in forearm volume, and venous return from the forearm was prevented by inflating the cuff at the upper arm; three to four consecutive arterial flow determinations were measured and averaged. A wrist cuff was inflated above systolic pressure 1 minute before flow determinations to occlude the blood circulation of the hand. Blood pressure was measured with a sphygmomanometer in the other arm, and forearm vascular resistance (mm Hg/ml/100 ml/min) was calculated by dividing mean blood pressure by forearm blood flow. Venous volume (ml/100 ml) was determined plethysmographically by the equilibration technique at the plateau of the volume curve, or, in any case, after 6 minutes of venous occlusion.

Variability of Plethysmographic Determinations

To assess the reproducibility of plethysmographic determinations of venous volume and forearm vascular resistance in our laboratory, plethysmography was performed twice, 1 hour apart, in seven control subjects; replicate measurements 24 hours apart were also obtained in another cohort of seven subjects. Replicate measurements of forearm vascular resistance yielded a mean difference of -1.16±0.75 mm Hg/ml/100 ml/min mean at 1 hour and -0.83±0.80 mm Hg/ml/100 ml/min at 24-hour determinations. Venous volume measurements showed a mean difference of -0.04±0.166 ml/100 ml at 1 hour and 0.23±0.17 ml/100 ml at 24-hour evaluations. Individual changes in venous volume or vascular resistance were considered significant only when exceeding 2 SD of 1-hour or 24-hour variability. We therefore considered "responder" to nitroglycerin and included in the study only 20 of 29 patients who had, at peak nitroglycerin titration, a venous volume increase of at least 0.33 ml/100 ml or a vascular resistance decrease of at least 1.5 mm Hg/ml/100 ml/min. Tolerance was defined after 24-hour infusion when a venous volume decrease of at least 0.34 ml/100 ml or a vascular resistance increase of at least 1.6 mm Hg/ml/100 ml/min was observed.

Protocol

After baseline measurements of heart rate, blood pressure, forearm blood flow, and venous volume, an intravenous infusion of nitroglycerin was started at a rate of 10 μg/min, and the rate was increased approximately every 5–10 minutes by 20–40 μg/min. The end point of nitroglycerin titration was a reduction in mean blood pressure of =10%; this was reached in an average of 50 minutes. At peak titration, all hemodynamic measurements were repeated. In patients who demonstrated dilation of venous and/or arterial peripheral vessels (changes in venous volume and/or vascular resistance higher than 2 SD of previously calculated 1-hour variability), the infusion was continued at constant rate. Plethysmography was performed again 24 hours after the beginning of infusion. Thirty minutes thereafter, all patients received a bolus administration of N-acetylcysteine (5 g in 200 ml 5% dextrose over 15 minutes), and the last determinations were obtained. Measurements and calculations of plethysmographic parameters were performed by a single investigator (R.P.) who was unaware of the sequence of the recordings.

Statistical Analysis

Data are expressed as mean±SD unless otherwise specified. Sequential measurements were compared using a repeated-measures ANOVA followed by Dunnett's multiple range test to differentiate among group means. Quantitative differences between groups were analyzed with the Student's t test for paired or unpaired data as necessary.

Results

Mean Blood Pressure and Heart Rate

Mean blood pressure decreased by an average 13% at peak nitroglycerin titration (from 90.8±13.8 to 79.2±9.0 mm Hg, p<0.01); at 24 hours, blood pressure increased to 83.2±8.7 mm Hg (p<0.05 versus peak titration). No changes occurred after N-acetylcysteine (83.2±9.6 mm Hg, NS versus 24 hours). Heart rate increased significantly at peak titration (from 68.7±9.1 to 77.3±11.9 beats per minute, p<0.01); at 24 hours, it was no more significantly different from baseline (69.4±11.0 beats per minute, NS versus control). After N-acetylcysteine, a slightly but nonsignificant change was observed (71.8±12.9 beats per minute, p=0.05–0.1). See Figure 1.

Forearm Venous Volume and Vascular Resistance

Nitroglycerin titration resulted in an average 26% increase in venous volume (from 2.69±1.08 to 3.42±1.22 ml/100 ml, p<0.01). However, at 24 hours, 48% of this increase was lost, and values were significantly lower than at peak titration (3.07±1.09 ml/100 ml, p<0.05). After N-acetylcysteine, venous volume increased again significantly, reaching values similar to those of peak titration (3.56±1.25 ml/100 ml, p<0.01 versus 24 hours). Forearm vascular resistance decreased by an average of 16% at peak nitroglycerin titration (from 29.8±12.6 to 24.9±11.3 mm Hg/ml/100 ml/min, p<0.01). Vascular resistance was slightly lower at 24 hours (23.1±8.8 mm Hg/ml/100 ml, NS versus peak titration) and did not change significantly after N-acetylcysteine (21.4±8.4 mm Hg/ml/100 ml/min, NS versus 24 hours). Forearm resistance data apply to 16 of 20 patients in whom a significant decline was observed after nitroglycerin titration. See Figure 2.

N-Acetylcysteine

To better define the effects of N-acetylcysteine, we evaluated the response to the sulfhydryl group donor separately in patients who developed and who did not develop tolerance in the arterial and venous beds.
Ten of 20 patients in whom venous volume at 24 hours was significantly lower than at peak nitroglycerin titration were considered as having "venous tolerance": In these patients, venous volume increased significantly after N-acetylcysteine (from 2.7±0.8 to 3.5±1.1 ml/100 ml, p<0.01), comparable to the peak titration level (3.5±1.3 ml/100 ml); a significant response to N-acetylcysteine was observed in all patients. In the remaining 10 patients, no change occurred after N-acetylcysteine (from 3.5±1.2 to 3.6±1.4 ml/100 ml, NS); in this subgroup, a significant response to N-acetylcysteine was observed in two of 10 patients (Figure 3). No significant effect of N-acetylcysteine was observed either in five of 16 patients in whom vascular resistance at 24 hours was significantly higher than at peak nitroglycerin titration (from 25.7±6.5 to 22.9±3.8 mm Hg/ml/100 ml/min, NS) or in 11 of 16 patients without arterial tolerance (from 21.9±9.7 to 20.8±9.9 mm Hg/ml/100 ml/min, NS).

**Nitroglycerin Infusion**

Mean dosage of nitroglycerin infusion was 169±115 μg/min. The dosage tended to be higher in patients who developed venous tolerance with respect to patients who did not develop venous tolerance (183±107 versus 155±122 μg/min, respectively; NS). On the contrary, the dosage tended to be lower in patients who developed arterial tolerance with respect to patients who did not develop arterial tolerance (117±71 versus 165±116 μg/min, respectively; NS).

**Discussion**

**Venous Versus Arterial Tolerance**

Despite the fact that many clinical studies have documented that long-term treatment with organic nitrates may lead to a decrease of at least some of their therapeutic efficacy, only in a few cases the venodilator effects of nitroglycerin have been directly measured and compared with the arterial effects of the drug. Zelis and Mason, in a plethysmographic study, have shown that during a 6–8-week treatment with isosorbide dinitrate, cross-tolerance develops to the venous but not to the arterial dilator effects of nitroglycerin. Manyari et al. found that during sustained therapy with isosorbide dinitrate, the adjunctive effects of sublingual nitroglycerin on both venous volume and blood pressure are markedly attenuated. The authors concluded that cross-tolerance between isosorbide dinitrate and nitroglycerin develops in both the arterial and venous systems. Different results were obtained in patients with congestive heart failure by Leier et al. Tolerance developed in their series only to the systemic arterial vascular effects.
Without attenuation of venous and pulmonary vascular effects. More recently, Makhoul et al.\textsuperscript{11} found in nine patients with congestive heart failure that the acute nitroglycerin effects attenuate more rapidly and more extensively during a 24-hour infusion in the venous than in the arterial circulation. Our results showing that after 24-hour continuous infusion nitroglycerin action is 50\% reduced in the venous circulation but not in the arterial circulation of the forearm confirm the observations by Zelis and Mason\textsuperscript{4} and are in accordance with more recent experimental data\textsuperscript{20} indicating that venous vessels are more readily susceptible to the development of nitrate tolerance than arterial vessels. It is of interest to note that a complete attenuation of nitroglycerin effects was found in the venous circulation of the 10 patients who showed venous tolerance as well as in the arterial circulation of the smaller cohort of patients with arterial tolerance (Figure 3). The discrepancy with the results obtained by Leier et al.\textsuperscript{10} is difficult to explain unless one hypothesizes that other mechanisms favoring a persistent decrease in preload, like increased renal perfusion and improved diuretic responsiveness,\textsuperscript{21} may take place during chronic vasodilator therapy in patients with congestive heart failure. Concerning the arterial effects of nitrates, it should also be stressed that the interpretation of the systemic hypotensive response to nitroglycerin in an individual patient is clinically difficult.

**Mechanisms of Arterial and Venous Tolerance**

Experimental studies have demonstrated that nitroglycerin causes relaxation of vascular smooth muscle by combining with intracellular compounds containing sulphydryl groups and producing S-nitrosothiols, which, in turn, activate the enzyme guanylate cyclase\textsuperscript{13}; the increase in cyclic GMP is the last step leading to smooth muscle relaxation. It has been suggested that sustained exposure to nitrates oxidizes the sulphydryl groups to a disulfide form with a reduced affinity for nitroglycerin.\textsuperscript{14} Exposure of tolerant rabbit aortic strips to the disulfide-reducing agent dithiothreitol makes them just as responsive as control strips.\textsuperscript{14} In the clinical setting, a systemic mechanism is also considered to play a major role in the development of nitrate tolerance: The decrease in intravascular pressures may trigger a rebound response of the sympathoadrenal axis and of the renin-angiotensin system that counteracts the peripheral effects of the drug, causing the return to baseline of left-sided and right-sided pressures. Our results confirm that venous tolerance is predominantly a consequence of the attenuation of nitroglycerin effects at the cellular level. In fact, the administration of the sulphydryl group donor \textit{N}-acetylcysteine caused a prompt reversal of venous tolerance in all patients who demonstrated a reduction of nitroglycerin venodilator effects at the end of the infusion period; furthermore, patients who developed venous tolerance tended to have higher infusion levels of nitroglycerin with respect to patients who did not develop venous tolerance, in accordance with the experimental data that cellular tolerance is a dose-dependent phenomenon.\textsuperscript{14} On the contrary, \textit{N}-acetylcysteine was seemingly not so effective in reversing arterial tolerance; these patients also received a lower dosage of nitroglycerin with respect to patients who did not develop arterial tolerance. The small number of patients in our study makes it impossible to draw any conclusions from these data. Previous studies\textsuperscript{6} have, however, suggested that arterial tolerance in humans is probably due to the reflex neurohormonal activation induced by the drug. The hypothesis that different mechanisms underlie arterial and venous tolerance to the same drug is not surprising. Experimental studies have demonstrated different intrinsic properties of arterial and venous endothelia,\textsuperscript{22} a more avid and more complete nitrate uptake in venous than in arterial vessels,\textsuperscript{23} and even differences in the threshold dosages necessary to induce vasodilatation in venous, arterial resistance, and capacitance vessels.\textsuperscript{24}

**Effects of \textit{N}-Acetylcysteine**

Our observations on the efficacy of \textit{N}-acetylcysteine in reversing venous tolerance and its lack of hemodynamic effects in nontolerant arterial and venous circulations are seemingly in contrast with previous studies indicating potentiation of nitroglycerin action with this sulphydryl group donor. Actually, much of this discrepancy might be attributed to differences in the protocol of \textit{N}-acetylcysteine administration. Pretreatment with \textit{N}-acetylcysteine potentiates the effects of a subsequent infusion of nitroglycerin: The increase in coronary flow\textsuperscript{25} or the decrease in systemic blood pressure and pulmonary capillary wedge pressure\textsuperscript{26} in response to a fixed dose of nitroglycerin was significantly higher after \textit{N}-acetylcysteine than under control conditions. However, in another study with protocol similar to ours, \textit{N}-acetylcysteine was added to a previous 24-hour infusion of nitroglycerin,\textsuperscript{27} only reversal of nitrate tolerance without potentiating effects was observed; however, these authors did not carry out a separate analysis in patients who developed and who did not develop nitrate tolerance. To further complicate the problem, only partial reversal of tolerance was observed by Packer and coworkers\textsuperscript{6} when \textit{N}-acetylcysteine was administered in eight nitrate-tolerant patients with congestive heart failure. Experimental studies are still unable, at the moment, to explain the conflicting results obtained in

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**Figure 3.** Graph shows changes in venous volume under control conditions, at peak nitroglycerin titration (peak T), after 24 hours of infusion (24 h), and after \textit{N}-acetylcysteine (NAC) in patients (pts) who developed tolerance (continuous line) and in patients who did not develop tolerance (dashed line). The F statistic for venous volume changes is 18.39 (p<0.001) for the tolerant group (n=10) and 11.53 (p<0.001) for the nontolerant group (n=10). See text for statistical significance of changes between 24 hours and NAC. Values are represented as mean±SEM.
human. The incubation of tolerant arterial vascular rings with different sulfhydryl donors produced reversed order of tolerance in most1,4,5 but not all20 studies; a nonspecific potentiation of nitroglycerin action has also been demonstrated,29 and it has been postulated to occur at an extracellular site.30 Clearly, one should take into account that the interactions between organic nitrates and sulfhydryl groups are so complex and still incompletely elucidated as to why different results have been obtained when different patients and different doses and schedules of N-acetylcysteine administration have been used in human studies.

Conclusions
The results of this study indicate that the susceptibility to the development of nitroglycerin tolerance in humans is higher in the venous than in the arterial circulation, and that venous tolerance can be rapidly reversed by the administration of a sulfhydryl group donor like N-acetylcysteine. We emphasize the fact that in clinical studies aimed at evaluating the attenuation of the beneficial action of nitrates and the possibility to reverse tolerance with the association of sulfhydryl group donors (or other compounds), a physiopathological interpretation of the data is impossible if we do not explore the effects of these drugs on both the arterial and venous sides of the circulation.

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References
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