Altered Peripheral Vasodilator Profile of Nitroglycerin During Long-Term Infusion of N-Acetylcysteine

SØREN BOESGAARD, MD, HELLE K. IVERSEN, MD, HENRIK WROBLEWSKI, MD, HENRIK E. POULSEN, MD, PhD, HANS FRANSEN, MD, JENS KASTRUP, MD, PhD, JAN ALDERSHVoY, MD, FsdD

Copenhagen, Denmark

Objectives. The aim of this study was to compare the short- and long-term effects of intravenous nitroglycerin plus placebo and nitroglycerin plus N-acetylcysteine on peripheral arteries, veins and microcirculation in humans.

Background. The thiol donor N-acetylcysteine may potentiate the hemodynamic response to nitrates in nitrate-intolerant and nonintolerant patients. The vascular changes responsible for this effect are not clear.

Methods. Eight male volunteers were treated with nitroglycerin (0.1 μg/kg per min) combined with N-acetylcysteine (2 g intravenously, followed by 5 μg/kg per h) or placebo for 23 h in a double-blind, randomized, crossover study. Venous volume, the diameter of the radial and temporal arteries, calf blood flow and subcutaneous blood flow were measured at baseline and repeated after 1 and 23 h of infusion.

Results. Prolonged coadministration of N-acetylcysteine and nitroglycerin potentiated the acute venodilator effect of nitroglycerin as estimated by changes in venous volume/nitroglycerin plus N-acetylcysteine, 4.4 ± 0.3 ml/100 g; nitroglycerin plus placebo, 3.65 ± 0.46 ml/100 g, mean ± SEM, p < 0.05; and prevented development of tolerance as seen after 23 h of treatment with nitroglycerin plus placebo (4.35 ± 0.6 vs. 3.47 ± 0.41 ml/100 g, p < 0.05). N-acetylcysteine had no effect on nitroglycerin-induced changes in arterial diameters (p > 0.05) but significantly increased microcirculatory subcutaneous blood flow after 1 h (nitroglycerin plus N-acetylcysteine: 6.3 ± 1.3 ml/100 mm Hg per min vs. nitroglycerin plus placebo: 3.5 ± 0.3 ml/100 mm Hg per min, p < 0.05) and after 23 h (4.4 ± 0.6 vs. 3.1 ± 0.5 ml/100 mm Hg per min, p < 0.05).

Conclusions. The results suggest that coadministration of nitroglycerin and N-acetylcysteine in humans potentiates and preserves nitroglycerin-induced venodilation and also augments the effect of nitroglycerin on small resistance vessels (regulating subcutaneous blood flow) without affecting the response to nitroglycerin in middle-sized arteries. Both the development of nitrate tolerance and the administration of N-acetylcysteine significantly change the normovascular profile of nitroglycerin in humans.

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Additions for correspondence: Dr. Søren Boesgaard, Medical Department B 2142, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark.
Methods

The study group comprised eight healthy male volunteers who were taking no medication; the mean age was 30 years (range 25 to 42). Electrocardiograms and physical examination were normal in all subjects. Informed consent was obtained from all participants, and the study was approved by the Scientific Ethical Committee of Copenhagen.

The investigation was performed as a double-blind, randomized, crossover trial. Period A consisted of 24 h of nitroglycerin infusion. N-acetylcysteine was confounded during the 1st 23 h. When the N-acetylcysteine infusion was terminated (at 23.5 h), a placebo bolus infusion was given. Period B consisted of 24 h of nitroglycerin infusion with placebo confounded during the 1st 23 h. When the placebo infusion was terminated, a bolus infusion of N-acetylcysteine was given. All subjects received treatments A and B in random order separated by a 1-week washout interval. Thus, the study consisted of a nitrate tolerance preventive period (the 1st 23 h) and a nitrate tolerance inteventional period (from 23 to 24 h). Nitroglycerin was administered as an intravenous infusion at a dose of 0.1 μg/kg per min. N-acetylcysteine confusion was given at a dosage of 2 μg/kg per min, followed by 5 mg/kg per h (in 0.9% saline solution). The bolus infusion of N-acetylcysteine consisted of 100 mg/kg (in 100 ml 0.9% saline solution) over 15 min. The nitroglycerin dose of 0.1 μg/kg per min was chosen because preliminary experiments showed that this dose induced significant changes in arterial diameters with only submaximal vasoconstriction, thus allowing detection of a further N-acetylcysteine-induced change in both of these investigative variables. The continuously infused dose of N-acetylcysteine has previously been reported (11) to modify tolerance development to the anti-ischemic effect of nitrates.

Studies were carried out in a warm quiet environment. Hemodynamic measurements were performed immediately before the start of infusions (pretreatment, 0 h, between 11 and 12 am) and repeated after 1 h (short-term), after 23 h (long-term) and after 24 h of nitroglycerin infusion (after the N-acetylcysteine/placebo bolus infusion). At each time point, measurements were performed after ≥2 h of supine rest.

Hemodynamic measurements. Venous volume measurement technique. Changes in venous tone were evaluated by measuring changes in venous volume (ml/100 g) by the phlebymographic equilibration technique, employing a mercury-in-Silastic strain gauge (10,17). The cuff was placed above the level of the heart and the strain gauge was placed at the calf site with the largest circumference. An ankle cuff was inflated to 200 mm Hg to isolate the foot from the circulation. A venous occlusion cuff was placed immediately below the knee and inflated to 30 mm Hg above the pressure where calf volume started to increase. The increase in venous volume reached a plateau after 3 to 4 min, and this value was considered to represent venous volume (ml/100 g calf) at each investigative time point.

Lower limb blood flow measurement technique. Subjects were instrumented as just described. The total arterial calf blood flow was calculated from the initial rate of change in calf volume occurring after a rapid inflation of the venous occlusion cuff (to approximately 50 mm Hg) and is reported as ml/100 g calf/min (18). Three to five flow determinations were measured and averaged. Calf vascular resistance was calculated by dividing mean arm arterial pressure by calf blood flow.

Systemic blood pressure and heart rate measurements. Systolic blood pressure, diastolic blood pressure and heart rate were measured every 15 min throughout the study period using an automatic, portable blood pressure recorder (Takeda Medical, A & D Co., Japan).

Subcutaneous blood flow measurement technique. Subcutaneous blood flow was measured approximately 10 cm above the knee on the lateral part of the thigh with the xenon-133 washout technique (19). The tracer (xenon-133) was applied epicutaniously and a portable semiconductor detector was fixed directly on the labeled skin surface by adhesive tape. The measurements were begun 30 min after the labeling procedure, allowing time for the lipophilic tracer to be deposited in the subcutaneous fat. Subcutaneous xenon-133 washout rate constants were recorded continuously in a portable data storage unit (Memollog System 600, Simonsen Medical, Randers, Denmark). Absolute subcutaneous blood flow (SBF) was calculated from: SBF = M x k x 100/min per 100 g, where M denotes the tissue to blood partition coefficient of xenon-133 (11 ml/g) in subcutaneous tissue. In the present situation, M may be considered to be constant throughout the study periods. Thus, changes in the washout rate constant (k), which is calculated from the xenon-133 disappearance curve, reflect microcirculatory changes in subcutaneous blood flow. Subcutaneous vascular resistance was calculated by dividing mean arterial pressure by the subcutaneous blood flow.

Arterial diameter measurement technique. Diameters of the temporal and radial arteries were measured with a high resolution, real time ultrasound technique (Dermacan C, Cortex Technology, Hadsund, Denmark). High frequency (20 MHz; bandwidth 15 MHz) ultrasound was directed perpendicular to the skin and the artery was located. Measurements were made at the right frontal branch of the superficial temporal artery and at the radial artery at the right wrist. Day to day positions of measurements were reproduced by recording the scanning position in relation to the angle and distance relative to the orthobasal line (temporal artery) and to the distal volar crest of the wrist (radial artery). To augment precision, data are presented as a mean of four measurements performed within 15 s. Details of this method have been described previously (20).

Statistics. Comparison of variables over time on each test day were performed by a repeated measures analysis of variance. Comparisons of the measured variables at each time interval on N-acetylcysteine and placebo days were made by Student's t test. Results are expressed as mean value
\[ z \text{ SEM. For all analyses, a } p \text{ value } < 0.05 \text{ was considered significant.} \]

**Results**

Vascular changes during continuous infusion of nitroglycerin and N-acetylcysteine. Baseline hemodynamic values at each investigative time point are presented in Table 1. None of the pretreatment values differed significantly between the two study periods (\( p > 0.05 \)).

<table>
<thead>
<tr>
<th>Pretreatment (0 h)</th>
<th>1 h</th>
<th>25 h</th>
<th>After Bolus Infusion (24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subcutaneous blood flow (mL/100 g per min)</strong></td>
<td><strong>P</strong></td>
<td><strong>NAC</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>3.0 ± 0.6</td>
<td>3.7 ± 0.3</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td><strong>NAC</strong></td>
<td>3.1 ± 0.3</td>
<td>3.3 ± 0.1*</td>
<td>3.2 ± 0.5*</td>
</tr>
<tr>
<td><strong>Radial artery diameter (mm)</strong></td>
<td><strong>P</strong></td>
<td><strong>NAC</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>2.82 ± 0.14</td>
<td>3.56 ± 0.13*</td>
<td>3.32 ± 0.26*</td>
</tr>
<tr>
<td><strong>NAC</strong></td>
<td>3.27 ± 0.11</td>
<td>3.49 ± 0.11*</td>
<td>3.04 ± 0.22</td>
</tr>
<tr>
<td><strong>Temporal artery diameter (mm)</strong></td>
<td><strong>P</strong></td>
<td><strong>NAC</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>1.14 ± 0.10</td>
<td>1.55 ± 0.13*</td>
<td>1.31 ± 0.06*</td>
</tr>
<tr>
<td><strong>NAC</strong></td>
<td>1.30 ± 0.08</td>
<td>1.63 ± 0.15*</td>
<td>1.35 ± 0.09*</td>
</tr>
<tr>
<td><strong>Vasovasomotor volume (mL/100 g)</strong></td>
<td><strong>P</strong></td>
<td><strong>NAC</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>3.52 ± 0.59</td>
<td>4.67 ± 0.46*</td>
<td>3.67 ± 0.40</td>
</tr>
<tr>
<td><strong>NAC</strong></td>
<td>3.44 ± 0.26</td>
<td>4.65 ± 0.36*</td>
<td>4.39 ± 0.25*</td>
</tr>
<tr>
<td><strong>Calf blood flow (mL/100 g per min)</strong></td>
<td><strong>P</strong></td>
<td><strong>NAC</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>2.54 ± 0.34</td>
<td>2.48 ± 0.29</td>
<td>2.84 ± 0.12</td>
</tr>
<tr>
<td><strong>NAC</strong></td>
<td>2.84 ± 0.35</td>
<td>2.94 ± 0.19</td>
<td>3.35 ± 0.32</td>
</tr>
<tr>
<td><strong>Mean arterial blood pressure (mm Hg)</strong></td>
<td><strong>P</strong></td>
<td><strong>NAC</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>85 ± 5</td>
<td>82 ± 3</td>
<td>84 ± 2</td>
</tr>
<tr>
<td><strong>NAC</strong></td>
<td>84 ± 3</td>
<td>84 ± 3</td>
<td>83 ± 2</td>
</tr>
<tr>
<td><strong>Heart rate (beats/min)</strong></td>
<td><strong>P</strong></td>
<td><strong>NAC</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>58 ± 3</td>
<td>70 ± 3*</td>
<td>66 ± 4*</td>
</tr>
<tr>
<td><strong>NAC</strong></td>
<td>57 ± 3</td>
<td>71 ± 5*</td>
<td>69 ± 5*</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \) compared with pretreatment values; \( p < 0.05 \) compared with placebo; \( p < 0.05 \) compared with nitroglycerin plus placebo was observed (Table 1, Fig. 2). Calf blood flow and vascular resistance. Neither nitroglycerin plus placebo nor nitroglycerin plus N-acetylcysteine infusion significantly influenced total calf blood flow (Table 1, Fig. 1). Because mean arterial pressure did not change during the study, the calculated calf vascular resistance was not significantly affected by either treatment. Heart rate and blood pressure. A small but significant \( p < 0.05 \) reduction in systolic blood pressure was observed after 1 h of infusion (nitroglycerin plus placebo, from 117 ± 2 to 114 ± 2 mm Hg; nitroglycerin plus N-acetylcysteine, from 119 ± 2 to 115 ± 6 mm Hg). These changes were maintained throughout the study period without any significant treatment difference (\( p > 0.05 \)). A trend toward reduced mean arterial blood pressure did not reach statistical significance (Table 1). Heart rate was significantly \( (p < 0.05) \) increased after 1 h (nitroglycerin plus placebo: from 58 ± 3 to 70 ± 3 beats/min; nitroglycerin plus N-acetylcysteine: from 57 ± 3 to 71 ± 5 beats/min, \( p < 0.05 \) (Table 1). Changes in heart rate were similar in both treatment periods and were maintained throughout the 24-h infusions.

Vascular changes after N-acetylcysteine bolus infusion. After intravenous nitroglycerin infusion for 2 h, after 23 h of nitroglycerin plus placebo infusion, a bolus infusion of N-acetylcysteine (100 mg/kg over 15 min) significantly increased calf blood flow (from 2.84 ± 0.12 mL/100 g per min to 3.21 ± 0.19 mL/100 g per min, \( p < 0.05 \) (Table 1, Fig. 3) and lowered calf vascular resistance (from 31.0 ± 0.72 to 27.1 ± 0.8 mm Hg/mL per 100 g/min, \( p < 0.05 \). When N-acetylcysteine was coadministered during the first 23 h of the 24-h nitroglycerin infusion, termination of N-acetylcysteine infusion and subsequent placebo bolus administration resulted in a significant decrease in calf blood flow (from...
3.35 ± 0.32 to 2.80 ± 0.25 mm Hg/ml per 100 g/min, p < 0.05 (Table 1, Fig. 3). N-acetylcysteine bolus administration significantly increased venous volume from 3.47 ± 0.41 to 4.26 ± 0.41 ml/100 g, p < 0.05 (Table 1, Fig. 3). For technical reasons, the effect of N-acetylcysteine bolus administration on subcutaneous blood flow was not investigated. Arterial diameters and other variables were not affected by N-acetylcysteine plus placebo bolus administration (p > 0.05) (Table 1).

Discussion

The present clinical study provides new information about the peripheral vascular effects of intravenous administration of N-acetylcysteine and nitroglycerin in the...
the effect on the venous vascular bed was lost, whereas an attenuated but significant effect on arterial diameters was still present. This finding is in agreement with previous reports (13,17,23) on both venous tolerance development and the rate of venous versus arterial tolerance appearance in patients without congestive heart failure, suggesting that within the 1st 24 h, tolerance to nitroglycerin develops predominantly on the venous side of the circulation. Thus, it is possible that the normal balance between nitroglycerin-mediated preload and afterload effects is changed during the development of tolerance in humans.

Effects of nitroglycerin plus N-acetylcysteine. The metabolism of nitroglycerin requires interaction with thiol such as cysteine and glutathione at one or more steps in the biotransformation to vasodilator nitrosothiols or nitric oxide (4,5,24). High doses of thiol donors, compounds (e.g., N-acetylcysteine) do not have intrinsic hemodynamic effects either in the coronary (6,15,36) or systemic (7,8,10,24,25) circulation. However, combined with nitroglycerin, thiol compounds significantly potentiate the vasodilator properties of nitroglycerin (6,10,15,16,34). At present, it is not known whether this interaction is due to a general potentiation of all the vasodilator effects of nitroglycerin or whether N-acetylcysteine modifies the vasodilator responses to nitroglycerin differently in various parts of the circulation. However, thiol administration to the coronary circulation of dogs partially enhances the effect of nitroglycerin in small resistance vessels but not normally sensitive to nitroglycerin, suggesting that thiol may change the vasodilator characteristics of nitroglycerin (5,16).

The present clinical results show that N-acetylcysteine also modifies the effect of nitroglycerin in microcirculatory vascular areas. This effect is revealed in the coronary circulation of healthy volunteers and is characterized by an increase in coronary blood flow (as measured by the xenon-133 washout technique). Because blood flow in this microcirculatory region is mainly regulated by precapillary small arterioles, the results strongly suggest that N-acetylcysteine, as in the coronary circulation, enhances nitroglycerin effects on small peripheral resistance vessels.

Total coronary blood flow decreased during the last hour of nitroglycerin infusion after termination of N-acetylcysteine infusion (Fig. 3, placebo), favoring some degree of N-acetylcysteine-induced flow change during the nitroglycerin plus N-acetylcysteine infusion period. This effect was, however, not reflected in measurements of total coronary blood flow at 1 and 23 h. Thus, despite the effect on subcutaneous blood flow, N-acetylcysteine concentrations (during infusion of 5 mg/kg per h) may be below the threshold for alterations of arterial tone in subcutaneous more important vascular beds; that is, skeletal muscle (preliminary data, unpublished observation). The finding that total coronary blood flow increases only after the N-acetylcysteine bolus administration (100 mg/kg per 15 min) may support this hypothesis and is compatible with a previous report (8) of a dose-dependent effect of thiol supplementation on nitroglycerin hemodynamics. Alterna-
tively, N-acetylcysteine-mediated effects on vascular resis-
tance may be subjected to powerful central or peripheral counterregulatory mechanisms (26).

The effect of continuous N-acetylcysteine infusion on the venous vascular bed includes a potentiation of the short-
term (1-3 h) response to nitroglycerin and a preservation of nitroglycerin effect throughout the study period. In addition, as previously reported (10,13), N-acetylcysteine bolus ad-
ministration increased venodilatation after development of venous tolerance. Both findings are in agreement with in vitro results of increased venous sensitivity to nitroglycerin after incubation with cysteine (12). Whether the effect of thiols compounds on nitrate responsiveness after the de-
velopment of tolerance represents a reversal of tolerance or a tolerance-independent nitrate/nitric oxide interaction is currently not clear. However, the effect of N-acetylcysteine bolus ad-
inistration is short-lived (14,27) and, from a practical point of view, preventive infusion of lower doses of thiols compounds may be a more rational approach.

Medium-sized arteries dilate in response to nitroglycer-
erin in vivo, but N-acetylcysteine administration appears to have no or minor influence on this response (13). An N-acetylcysteine-induced dilatation has previously been reported in the terminal artery in vivo, whereas dilatation of the radial artery (25) and large epineural coronary arterioles (26) seems unaffected by N-acetylcysteine adminis-
tration. In the current study, we were not able to detect any N-acetylcysteine-related potentiation of the nitroglycerin-
induced increase in radial and temporal artery diameters after short-term (1 h) and prolonged (23 h) exposure to nitroglycerin plus L-acetylcysteine or after N-acetylcysteine bolus ad-
inistration.

It is not known why the venous and arterial circulation may have different potentials for tolerance development and nitrate/nitric oxide interactions. There may be differences in the metabolic pathways (enzymes) and the ability of nitrate/nitric oxide compounds to induce nitric oxide. In addition, a differential arterial and venous response to nitroglycerin (and continu-
ous N-acetylcysteine infusion) is compatible with the con-
cept of a different vascular reactivity to nitroglycerin, de-
pending on the vascular basal nitric oxide sensitivity and nitric oxide production (29). According to this hypothesis, the amount of nitric oxide and nitrovasodilators produced from nitroglycerin may have a smaller hemodynamic potential in the arterial vascular bed because of decreased arterial sen-
sitivity to nitric oxide as compared with the venous circula-
tion. Assuming that tolerance follows reduced metabolic conversion of nitroglycerin to nitric oxide, tolerance may initially be detected in the vascular segments with the highest sensitivity to nitric oxide (thai is, veins). Further-
more, because of the significant basal nitric oxide production in arteries (but not in veins), the relative increase in arterial nitric oxide after N-acetylcysteine administration may be of less significance.

On the basis of this hypothesis, it is interesting to speculate that N-acetylcysteine potentiates the vascular effects of nitro-
glycerin by two mechanisms. The effect of a general "nonspe-
cific" mechanism (for example, extracellular thiols/nitric oxide interaction and nitric oxide production) depends on the sensitivity to nitric oxide in different vascular beds and consequently primarily augments venodilation, whereas only high doses of N-acetylcysteine and nitroglycerin may significantly influence vascular resistance. A "specific" mechanism involves dilatation of small resistance vessels only responsive to nitroglycerin in the presence of exogenous cysteine. This effect may specif-
ically enhance myocardial blood flow and increase the effects of nitroglycerin on afterload, thus changing the vasodilator profile of nitroglycerin.

The combined effect of these mechanisms is compatible with many of the published observations, which show that thiol supplementation, tolerance independently, may in-
crease the action of nitroglycerin on coronary blood flow (6,9,15,16), venous vascular volume (10) and systemic blood pressure (7,8,24). However, all data are consistent (29,30,31), and the significance of nitric oxide interactions probably depends on the experimental design, patient char-
acteristics, doses and schedules of drug administration. Clearly, the clinical implications of the thiol/nitrosyl interac-
tions are uncertain. Concern has been given to the possibility that coadministration of nitroglycerin and thiol may lead to an undesirable dilatation of small coronary resistance vessels, producing a coronary steal phenomenon in patients with ischemic heart disease. However, in this respect it may be relevant that continuous (as in the current study) or repeated intravenous administration of N-acetylcysteine for 24 h has previously been shown to potentiate nitroglycerin-induced anti-
ischemic effects in patients with unstable angina pectoris and to lower the incidence of acute myocardial infarction in pa-
ients with unstable angina pectoris, respectively (11,32).

Conclusions. This study provides the first data in humans on the peripheral hemodynamic and vascular effects of combined intravenous infusion of N-acetylcysteine and nitro-
glycerin in the prevention of nitrate tolerance. The results suggest that continuous infusion of N-acetylcysteine (1) po-
tentiates and preserves nitroglycerin-induced venodilatation, (2) increases subcutaneous blood flow mainly regulated by small precapillary resistance vessels, and (3) does not affect the response to nitroglycerin in middle-sized peripheral arterioles. Thus, continuous infusion of L-acetylcysteine en-
hances nitroglycerin-induced venodilatation and may moderate peripheral microcirculatory changes compatible with an al-
tered vasodilator profile of nitroglycerin.

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VASCULAR EFFECTS OF NITRATES AND NITRITE COMPOUNDS

E. K. BOSNJAČIĆ ET AL.

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