

N-acetylcysteine Improves Coronary and Peripheral Vascular Function

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OBJECTIVES	We investigated whether N-acetylcysteine (NAC), a reduced thiol that modulates redox state and forms adducts of nitric oxide (NO), improves endothelium-dependent vasomotion.
BACKGROUND	Coronary atherosclerosis is associated with endothelial dysfunction and reduced NO activity.
METHODS	In 16 patients undergoing cardiac catheterization, seven with and nine without atherosclerosis, we assessed endothelium-dependent vasodilation with acetylcholine (ACH) and endothelium-independent vasodilation with nitroglycerin (NTG) and sodium nitroprusside (SNP) before and after intracoronary NAC. In 14 patients femoral vascular responses to ACH, NTG and SNP were measured before and after NAC.
RESULTS	Intraarterial NAC did not change resting coronary or peripheral vascular tone. N-acetylcysteine potentiated ACH-mediated coronary vasodilation; coronary blood flow was $36 \pm 11\%$ higher ($p < 0.02$), and epicardial diameter changed from $-1.2 \pm 2\%$ constriction to $4.7 \pm 2\%$ dilation after NAC ($p = 0.03$). Acetylcholine-mediated femoral vasodilation was similarly potentiated by NAC ($p = 0.001$). Augmentation of the ACH response was similar in patients with or without atherosclerosis. N-acetylcysteine did not affect NTG-mediated vasodilation in either the femoral or coronary circulations and did not alter SNP responses in the femoral circulation. In contrast, coronary vasodilation with SNP was significantly greater after NAC ($p < 0.05$).
CONCLUSIONS	Thiol supplementation with NAC improves human coronary and peripheral endothelium-dependent vasodilation. Nitroglycerin responses are not enhanced, but SNP-mediated responses are potentiated only in the coronary circulation. These NO-enhancing effects of thiols reflect the importance of the redox state in the control of vascular function and may be of therapeutic benefit in treating acute and chronic manifestations of atherosclerosis. (<i>J Am Coll Cardiol</i> 2001;37:117-23) © 2001 by the American College of Cardiology

The endothelium is central to the regulation of vascular smooth muscle tone, hemostasis, inflammation and lipoprotein oxidation by generating nitric oxide (NO) (1). Endothelial dysfunction, associated with reduced NO bioavailability, is an early event in the pathogenesis of atherosclerosis (2). Atherosclerosis and its risk factors including hypercholesterolemia, hypertension and diabetes are associated with the generation of oxygen free radicals that degrade NO (3-5). Diminished NO activity, by upregulating nuclear factor kappa beta and expression of inflammatory cytokines and adhesion molecules (6), predisposes atherosclerotic lesions to vasoconstriction, increased platelet adhesion and aggregation and thrombus formation (2). Improving NO bioavailability may, therefore, reverse endothelial dysfunction, improve ischemia, reduce the risk of thrombotic complications and modify the progression of atherosclerosis.

Reduced thiols are molecules with a sulfhydryl group that have many biological functions, including scavenging oxygen free radicals, acting as cofactors for enzymatic reactions and modifying the half-life of NO by forming NO adducts (7). N-acetylcysteine (NAC), a thiol, is a pharmacological precursor of L-cysteine. When administered in its reduced

form, NAC rapidly increases systemic levels of cysteine (8). The properties of NO adducts (S-nitrosothiols) vary according to the thiol group. For example, S-nitrosocysteine has a short half-life but vasodilates and activates guanylate cyclase more potently than NO, whereas NO adducts of proteins may act as important stable reservoirs of NO (9-12). Hence, NO adducts appear to be the principal intermediates in the action of NO, and thiols may potentiate the activity of NO by either forming more biologically active adducts, by scavenging free radicals or by preventing NO oxidation and degradation.

Experimental studies have demonstrated that exogenous reduced thiols potentiate the effects of endogenous and exogenous NO (9,13,14). To determine whether exogenous thiols have similar effects in humans and, in particular, patients with atherosclerosis or its risk factors, we studied the effects of NAC in the human coronary and peripheral circulations. The effect of NAC on endogenous NO was studied by using the endothelium-dependent agonist acetylcholine (ACH), and the effect on exogenous NO donors was studied by using nitroglycerin (NTG) and sodium nitroprusside (SNP).

METHODS

Coronary vascular study. PATIENTS. We studied 16 patients, 7 with coronary atherosclerosis and 9 with angio-

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Manuscript received March 21, 2000; revised manuscript received August 15, 2000, accepted September 26, 2000.

Abbreviations and Acronyms

ACH = acetylcholine
 CV = coefficient of variation
 NAC = N-acetylcysteine
 NO = nitric oxide
 NTG = nitroglycerin
 SNP = sodium nitroprusside

graphically normal coronary arteries (Table 1), who were undergoing diagnostic cardiac catheterization for investigation of chest pain or abnormal noninvasive tests. There were nine (56%) men, and the mean age was 50 ± 11 years (Table 1). Patients with recent myocardial infarction, valvular heart disease or severe heart failure were excluded.

PROTOCOL. All cardiac medications were withdrawn at least 48 h before the study, and aspirin or other cyclooxygenase inhibitors were discontinued seven days before. The National Heart, Lung and Blood Institute's Institutional Review Board approved the study, and informed consent was obtained from all patients.

After diagnostic coronary angiography was performed, a 6F guide catheter was introduced into the proximal segment of a coronary artery, and blood flow velocity was measured using a 0.018 inch wire equipped with a Doppler crystal at its tip (Cardiometrics, Endosonics Corp., Rancho Cordova, California). The Doppler wire was advanced into either the left main or the proximal segment of a major epicardial artery free of significant stenosis (<30%). The wire tip was carefully positioned in a segment of the vessel that was straight and free of any major branches 1 cm from the tip that produced an adequate flow velocity signal and could be imaged without overlap from other vessels for quantitative measurements of the coronary artery diameter (15,16).

EFFECT OF NAC ON CORONARY VASCULAR RESPONSES TO ACH, NTG AND SNP. After a 5-min infusion of dextrose 5% at 1 ml/min, measurement of coronary blood flow velocity and coronary angiography were performed and repeated after each intervention. Endothelium-dependent vasodilation was estimated by performing a dose-response curve with 2-min incremental infusions of intracoronary ACH starting at 3 $\mu\text{g}/\text{min}$ in patients with atherosclerosis and at

30 $\mu\text{g}/\text{min}$ in those with normal coronary arteries. This regimen avoided excessive constriction that may occur at higher doses of ACH in atherosclerotic coronary arteries. The dose of ACH was not increased further once the infusion either reduced blood flow velocity or severely (>50%) narrowed the epicardial coronary tree. All patients received the 30 $\mu\text{g}/\text{min}$ concentration of ACH.

Endothelium-independent function was estimated with two NO donors; NTG was infused for 2 min each at 7.5 and 15 $\mu\text{g}/\text{min}$. After a 10-min period, SNP was infused at 20 and 40 $\mu\text{g}/\text{min}$ for 3 min each. After this, coronary flow reserve was measured with intracoronary adenosine administered at 2.2 mg/min for 2 min.

After a 15-min rest period and return of flow velocity to baseline values, an intracoronary infusion of NAC was started at 48 mg/min for 10 min. While continuing the NAC infusion, ACH was readministered at 30 $\mu\text{g}/\text{min}$, NTG at 7.5 and 15 $\mu\text{g}/\text{min}$, SNP at 20 $\mu\text{g}/\text{min}$ and adenosine at 2.2 mg/min for 2 min.

MEASUREMENT OF CORONARY BLOOD FLOW AND DIAMETER. Coronary blood flow was derived from the coronary blood flow velocity and diameter measurements using the formula ($\pi \times \text{average peak velocity} \times 0.125 \times \text{diameter}^2$). Coronary vascular resistance was calculated as mean arterial pressure \div coronary blood flow. For calculating flow, coronary artery diameter was measured in a 0.5-cm segment of vessel beginning 0.25 cm beyond the tip of the Flowire. Coronary angiograms were recorded using a cineangiographic system (Toshiba, Inc.), and quantitative angiography was performed with the ARTEK software (Quantim 2001, Statview, ImageComm Systems, Inc.). In addition to the measurement of the diameter at the level of the Doppler flow wire, 0.5-cm segments of mid- and distal regions of the epicardial coronary arteries were also measured by quantitative coronary angiography (15,16).

Because coronary epicardial vasodilation caused by NTG and NTP was not completely reversed by waiting 15 min after these drugs were given, we performed a separate analysis in segments of coronary arteries that returned to within $\pm 10\%$ of the baseline measurement. Coronary blood flow, however, returned to baseline 15 min after NTG and NTP.

REPRODUCIBILITY. The reproducibility of the coronary vascular responses to ACH was evaluated using two 30 $\mu\text{g}/\text{min}$ infusions of ACH in five patients. Coronary blood flow (183 ± 45 and 161 ± 35 mL/min, $p = 0.5$) and diameter (1.74 ± 0.2 and 1.73 ± 0.2 mm, $p = 0.5$, $n = 15$) were similar during the two infusions. The diameters were reproducible in the segments that dilated (2.12 ± 0.3 and 2.08 ± 0.3 mm, $n = 7$, $p = 0.4$) and those that constricted (1.42 ± 0.1 and 1.43 ± 0.1 mm, $n = 8$, $p = 0.5$) with the first infusion of ACH. All segments that constricted with the first infusion also constricted during the second infusion.

Femoral vascular study. PATIENTS. We studied 14 patients (12 of whom also underwent the coronary vascular

Table 1. Patient Characteristics

Variables	Study	
	Coronary	Femoral
Number	16	14
Men	10	9
Age, y	50.5 ± 3	48 ± 3
Cholesterol, mg/dl	201 ± 11	219 ± 12.4
HDL, mg/dl	38 ± 2	42 ± 3
Hypertension, n	3	3
Diabetes, n	2	3
Current smoking, n	5	5
Atherosclerosis, n	7	7

study >3 h before), seven with and seven without atherosclerosis of the coronary or the iliofemoral circulation. Their mean age was 48 ± 2.7 years; nine were men.

PROTOCOL. A 6F multipurpose A2 (Cordis, Inc.) catheter was introduced retrogradely 1 cm beyond the end of a 7F femoral artery sheath. A 0.018 inch Doppler Flowwire was introduced through the catheter and positioned 1 cm beyond the catheter tip to obtain an adequate flow velocity signal. All drugs were infused through the catheter 1 to 2 cm below the tip of the Doppler wire (17,18). A femoral angiogram was performed to assist with positioning of the wire and to visualize obstructive atherosclerotic plaques in the iliofemoral circulation, which may compromise blood flow measurements. Since diameter measurements were not made at the level of the Doppler wire with each intervention, we calculated the femoral vascular resistance index as the mean arterial pressure \div femoral blood flow velocity.

EFFECT OF NAC ON FEMORAL VASCULAR RESPONSES TO ACH, NTG AND SNP. After measurement of baseline flow velocity and mean arterial pressure, endothelium-dependent vasodilation was estimated by performing a dose-response curve with incremental infusions of ACH at 150 and 300 $\mu\text{g}/\text{min}$ for 2 min each. Endothelium-independent function was estimated with two NO donors; NTG at 25 $\mu\text{g}/\text{min}$ for 3 min and SNP at 20 $\mu\text{g}/\text{min}$ for 2 min.

After a 15-min recovery period, NAC was infused intraarterially at 48 mg/min for 10 min to achieve an estimated intraarterial concentration of 2 mol/liter. While continuing the infusion of NAC at 48 mg/min, ACH was coinfused at 150 $\mu\text{g}/\text{min}$ and at 300 $\mu\text{g}/\text{min}$ for 2 min each. After a 10-min recovery period, the NAC infusion was continued and coinfused with NTG at 25 $\mu\text{g}/\text{min}$ for 3 min and SNP at 20 $\mu\text{g}/\text{min}$ for 2 min. Blood flow velocity and mean arterial pressure were measured after each intervention.

REPRODUCIBILITY. Reproducibility of the microvascular dilator response to repeated infusions of ACH and SNP was tested in six patients during infusion of D5W. The femoral vascular resistance indexes during the first and second infusions were as follows: ACH (150 $\mu\text{g}/\text{min}$), 3.5 ± 0.6 and 4.0 ± 0.6 , $p = 0.2$ (coefficient of variation [CV] 17.2%); ACH (300 $\mu\text{g}/\text{min}$), 3.1 ± 0.5 and 3.7 ± 0.6 , $p = 0.2$ (CV 8.2%); SNP, 1.8 ± 0.1 and 1.8 ± 0.2 , $p = 0.7$ (CV 8.7%). These findings are consistent with our previously described reproducibility of vascular responses in the femoral circulation (17,18).

Statistical analysis. Data are expressed as mean \pm SEM. Differences between means were compared by paired or unpaired Student t -test, as appropriate. The effect of NAC on the effect of NTG in the coronary circulation and of ACH in the femoral circulation were compared by two-way analysis of variance for repeated measures with drug and dose as main effects and drug X dose interaction (Sigmastat, Version 1.0). If the F value was significant, a Bonferroni multiple comparison test was performed. All p values were

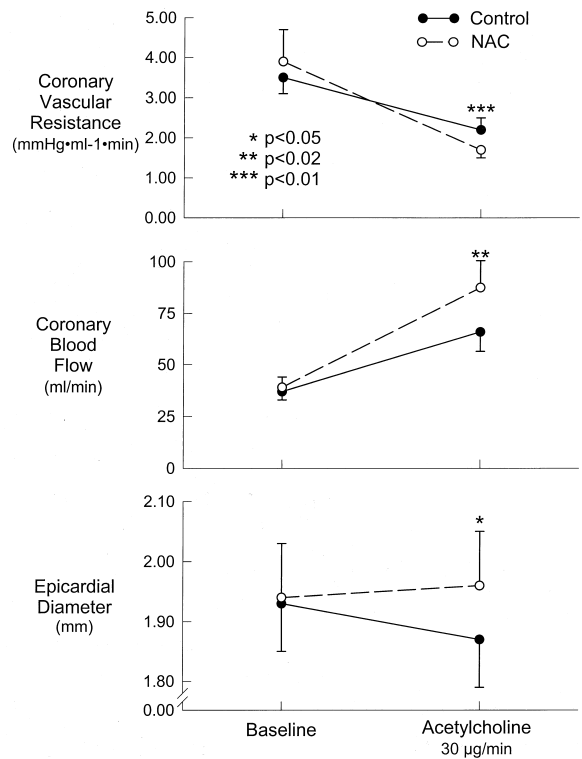


Figure 1. Change in coronary vascular resistance, coronary blood flow and epicardial diameter in response to acetylcholine before (control) and after N-acetylcysteine (NAC). P values compare control versus NAC.

two-tailed, and a value <0.05 was considered to be of statistical significance.

RESULTS

Coronary microcirculation. EFFECT OF NAC ON THE RESPONSES TO ACH. After 10 min of NAC infusion, heart rate (75 ± 3.1 to 75 ± 3.0 beats/min, $p = 0.9$) and mean arterial pressure (109 ± 4.1 to 109 ± 3.8 mm Hg, $p = 0.5$) remained unchanged. There was also no change in coronary blood flow (41.1 ± 4.8 to 39.3 ± 5.1 ml/min, $p = 0.3$) or coronary vascular resistance (3.2 ± 0.4 to 3.9 ± 0.8 mm Hg \cdot ml $^{-1}$ \cdot min, $p = 0.2$).

N-acetylcysteine augmented ACH-mediated microvascular dilation, indicating significant potentiation of endothelium-dependent vasomotion. Compared with baseline responses with 30 $\mu\text{g}/\text{min}$ ACH, coronary vascular resistance was $21 \pm 6\%$ lower, $p < 0.01$ (Fig. 1). This potentiating effect of NAC was observed in patients with normal coronary arteries (vascular resistance fell from $-41 \pm 7\%$ to $-54 \pm 8\%$, $p = 0.01$), and those with atherosclerosis (vascular resistance changed from $-22 \pm 12\%$ to $-41 \pm 10\%$, $p = 0.07$).

EFFECT OF NAC ON THE RESPONSE TO SNP. Sodium nitroprusside produced graded microvascular dilation that was potentiated by NAC. Thus, compared with baseline, NAC produced a further 68% increase in coronary blood flow ($p < 0.01$) at the 20 $\mu\text{g}/\text{min}$ dose (Fig. 2).

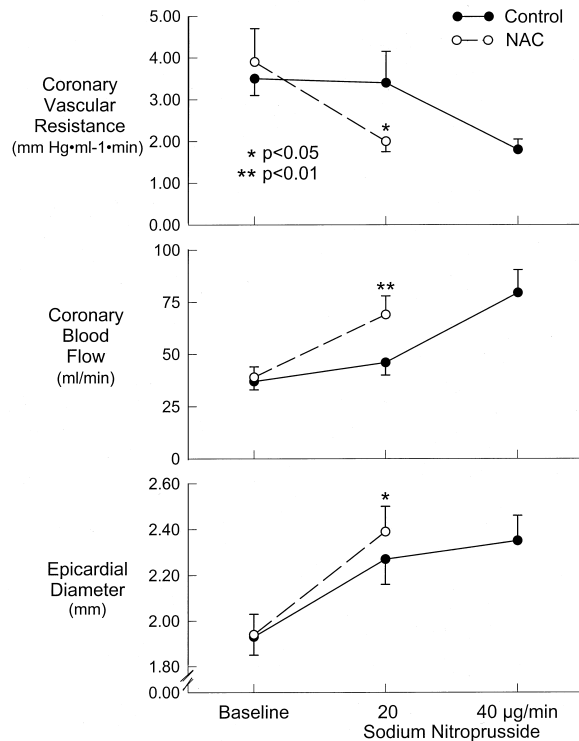


Figure 2. Change in coronary vascular resistance, coronary blood flow and epicardial diameter in response to sodium nitroprusside before (control) and after N-acetylcysteine (NAC).

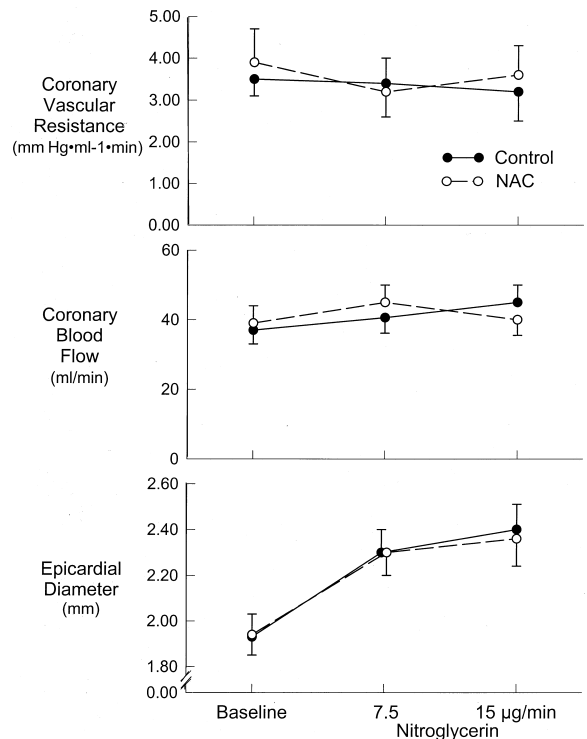


Figure 3. Change in coronary vascular resistance, coronary blood flow and epicardial diameter in response to nitroglycerin before (control) and after N-acetylcysteine (NAC).

EFFECT OF NAC ON THE RESPONSE TO NTG. Nitroglycerin did not alter resting coronary blood flow or vascular resistance, and NAC did not affect responses to NTG (Fig. 3).

EFFECT OF NAC ON THE RESPONSE TO ADENOSINE. Intracoronary adenosine produced microvascular vasodilation that was also unaffected by NAC. The baseline increase in coronary blood flow of $366 \pm 33\%$ remained unchanged after NAC ($387 \pm 57\%$, $p = \text{NS}$, respectively; Fig. 4).

Coronary epicardial response to NAC. In the group as a whole, there was a mild residual vasodilation in the 46 epicardial coronary segments measured 15 min after the administration of NTG, NTP and adenosine (from 1.95 ± 0.1 to 2.15 ± 0.1 mm, $p < 0.01$). To overcome the effect of this baseline shift, we examined the effects of ACH, NTP and NTG on 31 epicardial segments that returned to their baseline diameter (1.92 ± 0.1 before and 1.94 ± 0.1 after an NAC).

Acetylcholine ($30 \mu\text{g}/\text{min}$) produced a $1.4 \pm 2\%$ constriction in mean epicardial vessel diameter at baseline. After NAC, this was improved to $4.7 \pm 2\%$ dilation, $p = 0.033$ (Fig. 1). Similarly, epicardial dilation in response to SNP ($20 \mu\text{g}/\text{min}$) was improved by NAC: $17.5 \pm 3\%$ before to $24.7 \pm 3\%$ after NAC, $p = 0.03$ (Fig. 2). In contrast, NTG-mediated epicardial dilation at the 7.5 and $15 \mu\text{g}/\text{min}$ concentrations remained unchanged after NAC ($19.4 \pm 3\%$ to $20.1 \pm 3\%$, $p = \text{NS}$) before versus after NAC, respectively, at the low dose of NTG (Fig. 3).

Effect of NAC on femoral microcirculation.

N-acetylcysteine infusion did not alter resting arterial pressure or femoral microvascular tone; femoral vascular resistance index was 5.8 ± 0.6 before and 5.1 ± 0.6 mm Hg·m⁻¹·s, $p = 0.8$ after NAC. Acetylcholine produced progressive vasodilation of the femoral microcirculation that was significantly potentiated by NAC, $p = 0.001$ by analysis of variance (Fig. 5). This effect was greater at the lower dose

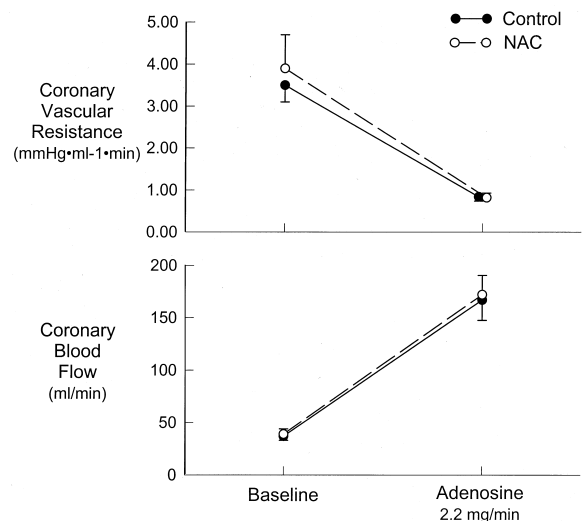


Figure 4. Change in coronary vascular resistance and coronary blood flow in response to intracoronary adenosine before (control) and after N-acetylcysteine (NAC).

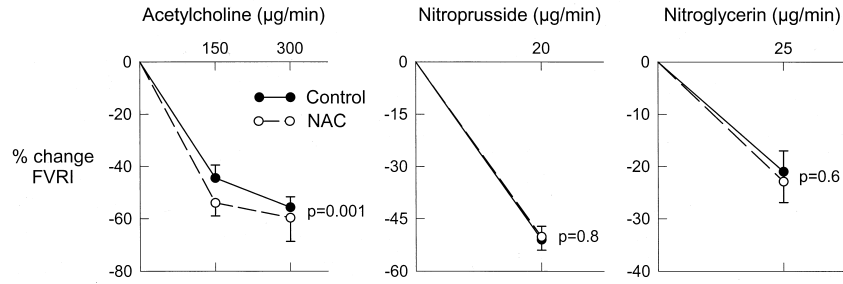


Figure 5. Change in femoral vascular resistance index (FVRI) with acetylcholine (left panel), sodium nitroprusside (middle panel) and nitroglycerin (right panel) before (control) and after N-acetylcysteine (NAC). P values with and without NAC are compared by analysis of variance.

of ACH. Improvement was observed in patients with normal and depressed baseline responses to ACH.

Unlike the coronary circulation, SNP-mediated femoral vasodilation was not enhanced by NAC (Fig. 5). Similar to the coronary circulation, no increase in NTG-induced vasodilation was observed in the femoral microcirculation after NAC (Fig. 5).

DISCUSSION

The three major findings of this study were: 1) NAC, a reduced thiol, improves endothelium-dependent vasomotion in the coronary and peripheral circulations of patients with and without atherosclerosis, 2) NAC potentiates SNP-mediated coronary, but not femoral, vasodilation and 3) NAC did not potentiate NTG-mediated coronary or femoral vasodilation.

Effect of NAC on endothelium-dependent vasodilation.

Another reduced thiol, glutathione, also improves endothelial dysfunction in atherosclerosis by enhancing stimulated NO activity (19,20). In this study, we show that NAC has no effect on basal vasomotor tone but improves endothelium-dependent responses. This effect was observed in patients with and without endothelial dysfunction or atherosclerosis.

Potential mechanisms underlying the improvement in endothelial function with NAC.

N-acetylcysteine may enhance the bioavailability of NO by spontaneously forming S-nitroso-N-acetylcysteine and a stable, biologically active transnitrosation byproduct, S-nitrosocysteine (21,22). Though NO has a very short half-life, in the order of 0.1 to 1 s, evidence suggests that it circulates in the plasma primarily as S-nitrosoalbumin after reacting with the sulfhydryl group of cysteine 34 and in red blood cells as nitrosyl-hemoglobin and S-nitrosohemoglobin (21–23). S-nitrosoalbumin is biologically active but has minimal intracellular access and probably serves as a circulating reservoir of NO (23). Low molecular weight thiols, such as cysteine, form less diffusion-limited NO adducts that may transport NO to target sites within vascular smooth muscle cells and platelets (21).

The second potential mechanism for action of NAC may relate to its antioxidant properties (11,12,14). Increased generation of oxygen free radicals, largely responsible for

inactivating NO (3–5,24), may be scavenged from plasma or endothelial cells by NAC, thereby increasing NO bioavailability (3–5,24–26). Support for this mechanism is provided by the observation that NAC does not potentiate endothelium-dependent vasodilation in normal volunteers in whom oxidative stress is low (27).

The effect of NAC on exogenous NO donors. Nitroglycerin requires enzymatic metabolism in the cell membrane of vascular smooth muscle cells to liberate NO (28). Thus, exogenously administered thiols would need to elevate vascular interstitial levels to potentiate NTG responses. In our study, NTG-mediated microvascular vasodilation was not potentiated by NAC, contrary to previous studies in canine or human circulation (29–33). These differences may be partly due to the longer duration of NAC administration (27,31) or its intravenous, as opposed to intraarterial, delivery (31,33) in previous studies that may have altered the bioavailability of NAC. Finally, Creager and associates (27) studied normal subjects, whereas the majority of our patients had atherosclerosis or its risk factors.

Sodium nitroprusside releases NO by an endothelium-independent mechanism in the presence reducing agents such as thiols that are present in plasma and tissue extracts (34,35). A previous human study demonstrated no potentiation of systemic or pulmonary hemodynamic effects of SNP with NAC (36), a finding that is in agreement with our results in the femoral microcirculation. Our study, however, demonstrates that NAC augments SNP-mediated coronary epicardial and microvascular dilation, suggesting that thiols, such as cysteine, contribute to more rapid generation of NO from SNP in the human coronary circulation.

Study limitations. Because there is no current method to administer NO directly into the coronary circulation, we were unable to directly assess the effects of NAC on NO. Even if this had been achieved, exogenous NO would instantly form adducts with circulating endogenous thiols. We also cannot exclude the unlikely possibility that enhancement of ACH responses by NAC was independent of NO, but we recently demonstrated that the improvement with another thiol, glutathione, is mediated through increased NO bioavailability (19).

Caution should be advised in interpreting the effects of

NAC on the coronary epicardial vessels because epicardial diameter did not return to baseline in some patients after NTG and NTP were administered. To overcome this, we analyzed the effects of repeat administration of the agonists after NAC only in those patients in whom epicardial diameters returned to baseline. We may have missed improvement in the NTG responses because of near maximal epicardial vasodilation observed at the doses employed. However, augmentation in SNP responses was detectable with vasodilation at baseline similar to that of NTG.

Conclusions and implications. In this study, we demonstrate that coronary endothelium-dependent and SNP-mediated vasodilation is improved by NAC. This acute improvement in endothelial function and the recent demonstration that S-nitrosoglutathione has platelet inhibitory effects in humans indicate that thiols such as NAC and glutathione may have therapeutic potential (19,37,38). The combination of NTG and NAC significantly reduced the incidence of myocardial infarction compared with NTG alone in patients with severe unstable angina (39). Furthermore, an inhibitory effect of S-nitrosoalbumin on neointimal proliferation and of L-2-oxothiazolidine-4-carboxylic acid, a cysteine prodrug, on improving endothelial dysfunction, suggests that long acting NO-adducts may have antiatherogenic properties (40,41).

Acknowledgments

The authors would like to thank Gloria Zalos, RN, William H. Schenke, BS, and Rita Mincemoyer, RN, for their technical assistance.

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