Potentiation of Isosorbide Dinitrate Effects With N-Acetylcysteine in Patients With Chronic Heart Failure

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Background Supply of sulfhydryl groups with the administration of N-acetylcysteine (NAC) has been reported to reverse tolerance to nitroglycerin but not to isosorbide dinitrate (ISDN). Lack of interaction between NAC and ISDN was suggested as an explanation for these findings. The present study was therefore designed to further evaluate this hypothesis. For this purpose, we compared the hemodynamic and hormonal effects of ISDN when given alone and in combination with NAC.

Methods and Results We performed a randomized, crossover design evaluation of the hemodynamic and hormonal effects of ISDN and ISDN+NAC in 14 patients with chronic congestive heart failure due to left ventricular systolic dysfunction. The findings of this study demonstrated a substantial NAC-mediated potentiation of ISDN effect on mean right atrial pressure (-11±21% versus -38±27%, -17±20% versus -34±27%, and -7±20% versus -25±26% at 2, 3, and 4 hours, respectively; all P<.05), mean pulmonary artery wedge pressure (-18±16% versus -33±14%, -15±25% versus -33±19%, -14±22% versus -25±22%, and -16±16% versus -26±16% at 2, 3, 4, and 5 hours, respectively; all P<.05), mean pulmonary artery pressure (-8±11% versus -20±15% at 3 hours, P<.05), and cardiac output (an increase of 2±16% versus 25±20% at 4 hours, P<.05). Although there were no significant changes in serum catecholamine levels and plasma renin concentration with both regimens, ISDN+NAC resulted in a greater fall in plasma levels of atrial natriuretic peptide (296±251 pg/mL after ISDN versus 202±118 pg/mL after ISDN+NAC, P<.05).

Conclusions The results of this study provide strong evidence for the existence of an interaction between thios and ISDN and further support the role of sulfhydryl groups in the activation and therapeutic action of organic nitrates. The discrepancy between the results of this study demonstrating NAC-induced potentiation of ISDN effects and a previous study showing failure to reverse ISDN tolerance with NAC may suggest that ISDN-NAC interaction requires normal intracellular levels of sulfhydryl groups and does not occur after intracellular sulfhydryl group depletion. (Circulation. 1994;89:2595-2600.)

Key Words: • thios • nitroglycerin • isosorbide dinitrate

The vasodilatory effect of organic nitrates has been long recognized as a main cause for their therapeutic benefit.1 There is substantial evidence suggesting that the pharmacological effect of these drugs depends on their cellular conversion to S-nitrosothiols and nitric oxide, which activate the enzyme guanylate cyclase, leading to intracellular accumulation of cyclic guanosine monophosphate (cGMP).2 The activation of organic nitrates appears to be dependent on the presence of sulfhydryl groups, which are derived from intracellular cysteine.3-5 This thiols-nitrates interaction has been supported by augmentation of nitrate effect with sulfhydryl groups containing compounds such as N-acetylcysteine (NAC) and methionine, which has been demonstrated both in vitro and in vivo.5-9 Depletion of sulfhydryl groups also has been postulated as a possible mechanism responsible for the development of nitrate tolerance with continuous nitrate therapy.10-12 Reversal of tolerance and partial restoration of nitroglycerin (NTG) effect with the administration of NAC have been documented by a number of investigators.11-13 At the same time, however, the administration of NAC failed to reverse tolerance to the antianginal effect of isosorbide dinitrate (ISDN).14 In an attempt to explain the difference between thiol effect on tolerance to NTG and ISDN, Fung et al15 proposed an extracellular interaction between sulfhydryl groups and NTG not occurring with ISDN due to a much slower rate of NAC-catalyzed production of S-nitrosothiol. The present study investigated in a randomized, crossover fashion the hemodynamic and hormonal effects of ISDN alone and in combination with NAC in a group of patients with chronic congestive heart failure (CHF) in an attempt to further evaluate the presence or absence of an interaction between exogenous thios and ISDN.

Methods

Study Population We studied 14 patients with severe chronic CHF (New York Heart Association functional classes III and IV) due to left ventricular (LV) systolic dysfunction who were admitted to the hospital for worsening symptoms. There were 12 men and 2 women ranging in age from 20 to 67 years (mean±SD, 51±14 years). The cause of CHF was suspected to be coronary artery disease in 1 patient and dilated cardiomyopathy in 13 patients. Of the patients with dilated cardiomyopathy, a history of excessive alcohol consumption was obtained in 7 patients. None of the patients had evidence of acute myocardial isch-
emia at the time of the study, and all patients were in stable clinical and hemodynamic condition. The diagnosis of LV systolic dysfunction was confirmed by contrast or radionuclide ventriculography in 7 patients who demonstrated LV ejection fraction ranging from 12% to 28% (mean±SD, 19±5%). In the remaining 7 patients, LV systolic dysfunction was confirmed by echocardiography (fractional shortening ranging between 5% and 19%; mean±SD, 13±5%).

**Hemodynamic Measurements and Computations**

Hemodynamic measurements were obtained with the use of a balloon-tipped, triple-lumen, right heart flotation catheter (Swan-Ganz). Right atrial, pulmonary arterial, and pulmonary artery wedge pressures were recorded on an Electronics for Medicine AR-6 recorder, and mean pressures were obtained with the use of electronic integration. Heart rate was determined from the ECG recordings, and arterial blood pressure was measured by the standard cuff method. Cardiac outputs were obtained by the thermodilution method as previously described. Mean systemic arterial pressure, cardiac index, systemic vascular resistance, and pulmonary vascular resistance were calculated by standard formulas.

**Study Protocol**

All patients were enrolled in the study after being clinically stabilized and signing a consent form. Evaluation began at least 24 hours after insertion of a right heart balloon flotation catheter to guard against previously reported spontaneous hemodynamic fluctuations after the procedure. Vasodilator agents including organic nitrates were discontinued at least 24 hours before initiation of the study. Usual doses of digitalis and oral furosemide were continued throughout the study period. To ensure hemodynamic stability, baseline hemodynamic measurements were obtained every 30 minutes to obtain two consecutive measurements with <10% variability in mean pulmonary artery wedge pressure. Hemodynamic values obtained at the last measurements were used as baseline values. After the determination of baseline hemodynamic and hormonal values, patients were randomized to receive 40 mg of oral ISDN (11 patients) or 120 mg (in 3 patients not responding to the smaller dose) either alone or after intravenous infusion of NAC (Bristol-Myers Squibb Corporation). Hemodynamic measurements were repeated hourly for 6 hours, and determination of plasma hormonal values was repeated 2 hours after ISDN administration. A washout period of at least 24 hours was allowed after the administration of the first dose of ISDN to achieve a return of hemodynamic and hormonal values to baseline. All patients were then crossed over to receive the second regimen. Seven patients received oral ISDN alone as their first regimen; the other 7 patients received ISDN after an infusion of NAC.

**ISDN+NAC Administration**

NAC was administered intravenously via a peripheral vein in an infusion of 100 mg/kg body wt diluted in 200 mL of 5% dextrose solution given over 30 minutes followed by administration of 40 to 120 mg of oral ISDN. All patients were monitored closely throughout the study period for any adverse effects to administration of intravenous NAC. Patients with a history of allergy to sulfur-containing substances or chronic obstructive pulmonary disease were excluded from the study.

**Plasma Neurohormonal Measurements**

Venous blood samples for plasma catecholamines, renin activity, and atrial natriuretic peptide (ANP) were obtained at baseline each day and 2 hours after the administration of ISDN alone and in combination with NAC. The determination of both epinephrine and norepinephrine was performed with an isotope radioenzymatic technique. Plasma renin activity was determined by radioimmunoassay of angiotensin I after addition of excess sheep angiotensinogen. ANP was measured by radioimmunoassay technique (RIA kit, TRK.500, Amersham Corporation). Determinations of plasma neurohormonal values were performed by outside laboratories in a blinded fashion.

**Statistical Methods**

The temporal changes in hemodynamic and hormonal values were analyzed within each treatment group by ANOVA for repeated measurements and the Newman-Keuls test. All data comparisons between the two groups were made by two-tailed paired t test. A probability value of <.05 was considered statistically significant. All group values were presented as mean±SD. Analysis was performed with the CLINFO system and SAS statistical package on an IBM 370 system at the University of Southern California.

**Results**

**Comparison of Baseline Hemodynamic and Hormonal Values in Study Days 1 and 2**

Baseline data as determined before drug administration on both study days showed no statistically significant difference between any of the hemodynamic or hormonal values (Tables 1 and 2).

**Acute Hemodynamic Response to ISDN vs ISDN+NAC**

NAC potentiated the effect of ISDN on right atrial pressure, mean pulmonary artery wedge pressure, mean...
pulmonary artery pressure, and cardiac output. The total values of these parameters as measured throughout the study period are shown in Table 1. Despite comparable values at baselines, mean right atrial pressure was significantly lower after the administration of ISDN+NAC when compared with ISDN alone at 2 hours (8±6 versus 12±6 mm Hg, *P*<.01), 3 hours (9±6 versus 12±7 mm Hg, *P*<.01), and 4 hours (10±6 versus 13±8 mm Hg, *P*<.05). Mean pulmonary artery wedge pressure was significantly lower on ISDN+NAC at 2, 3, and 5 hours (20±7 versus 24±9, 20±8 versus 25±9, and 21±7 versus 24±8 mm Hg, respectively). Similarly, mean pulmonary artery pressure was significantly reduced after ISDN+NAC compared with ISDN alone at 3 hours (35±9 versus 40±10 mm Hg, *P*<.01). Cardiac output values were comparable before administration of both regimens but were significantly higher after ISDN+NAC at 2 hours (4.1±1.3 versus 3.6±1.0 L/min, *P*<.05), 3 hours (4.3±1.3 versus 3.3±0.8 L/min, *P*<.01), 4 hours (3.8±1.2 versus 3.3±1.1 L/min, *P*<.05), and 5 hours (4.0±1.3 versus 3.4±1.0 L/min, *P*<.05).

Analysis of individual hemodynamic changes from baseline are shown in Figs 1 through 4. Mean right atrial pressure showed a similar reduction at 1 hour with both ISDN (−31±20%) and ISDN+NAC (−37±26%, *P* = NS). However, the effect was markedly longer with ISDN+NAC, as indicated by a significant difference at 2 hours (−11±21% versus −38±27%), 3 hours (−17±20% versus −34±27%), and 4 hours (−7±20% versus −25±26%). Similarly, ISDN+NAC had a greater effect on mean pulmonary artery wedge pressure (Fig 2) when compared with ISDN alone (−18±16% versus −33±14% at 2 hours, −15±25% versus −33±19% at 3 hours, −14±22% versus −25±22% at 4 hours, and −16±16% versus −26±16% at 5 hours, all *P*<.05). The difference in the effect of the two regimens on mean pulmonary artery pressure (Fig 3) showed a similar trend but achieved statistical significance only at 3 hours (−8±11% versus −20±15%, *P*<.05). The augmentation of cardiac output (Fig 4) was more pronounced with ISDN+NAC, with a significant difference at 3 hours (2±16% versus 25±20%). Both ISDN and ISDN+NAC resulted in a slight and a similar fall in blood pressure (−11±10% and −12±9%, respectively, at its maximum). Similarly, no significant difference was noted between the effect of ISDN or ISDN+NAC on pulmonary and systemic vascular resistance.

**Hormonal Changes to ISDN vs ISDN+NAC**

Table 2 demonstrates the neurohormonal levels at baseline and 2 hours after administration of ISDN alone and ISDN+NAC. Despite significant hemodynamic potentiation of effects of ISDN+NAC, there were no statistically significant changes in plasma levels of epinephrine, norepinephrine, and renin. ANP changed only slightly and insignificantly after the administration of ISDN alone but demonstrated a substantial reduction after the administration of NAC+ISDN. There was no significant difference between ANP values measured at both baselines and after the administration of ISDN alone. However, mean ANP value 2 hours after ISDN+NAC was 202±113 pg/mL and was significantly lower than values at baseline before ISDN (316±240 pg/mL, *P*<.05) and after ISDN alone (296±251 pg/mL, *P*<.05).

**Discussion**

The results of the present study demonstrate a marked augmentation of the vasodilatory effect of oral ISDN after the administration of NAC in patients with chronic CHF. Exogenous supply of sulfhydryl groups...
potentiated the reduction in both right and left ventricular filling pressures and mean pulmonary arterial pressure and the increase in cardiac output. These hemodynamic changes were associated with a larger fall in ANP levels. Since a strong relation between plasma ANP levels and right and left ventricular filling pressures has been demonstrated previously in patients with CHF, the difference in response of these parameters to therapy is most likely the cause for a larger change in ANP after combination therapy with ISDN+NAC.

It has been suggested that availability of sulfhydryl groups is critical for the cellular biotransformation of organic nitrates and the formation of vasoactive compounds. This suggestion has been supported by studies in vitro and in vivo demonstrating augmentation as well as reduction of nitrate effects by sulfhydryl groups enhancing or depleting compounds, respectively. Failure of sulfhydryl groups supply to reverse tolerance to the antischismic effect of ISDN in patients with angina pectoris raised a doubt regarding the existence of thiol-ISDN interaction. Our findings, however, provide strong evidence for the existence of such an interaction, which may have important clinical implications in augmenting the therapeutic effect of ISDN as well as preventing or reversing nitrate tolerance.

There are a number of clinical reports demonstrating the enhancement of nitrate effects with sulfhydryl groups supplementation. Horowitz et al. first demonstrated NAC-induced augmentation of the hemodynamic effects of NTG in patients with coronary artery disease and later showed enhancement of NTG clinical efficacy in preventing myocardial infarction in patients with unstable angina. Winniford et al. showed potentiation of NTG-induced coronary dilatation by NAC in patients undergoing cardiac catheterization for evaluation of chest pain; Levy et al. showed augmentation of

the acute hemodynamic effect of NTG by methionine, a precursor of cysteine, in patients undergoing cardiac catheterization for evaluation of chest pain; and most recently, Meredith et al. reported potentiation of NTG effect in the coronary bed in patients with ischemic heart disease with captopril, a sulfhydryl group containing angiotensin-converting enzyme inhibitor. The present study evaluating patients with chronic heart failure demonstrates that thiol interaction with organic nitrates is not limited to NTG alone but occurs with ISDN as well. The ability of sulfhydryl groups supply to augment ISDN effect is further supported by a recent study demonstrating a marked augmentation of the anti-ischmic effect of oral ISDN in patients with angina pectoris with a concomitant administration of captopril.

Nitrate tolerance often results in a marked, early attenuation of nitrate effect. Although the mechanism of this phenomenon is not entirely clear and may be multifactorial, depletion of sulfhydryl groups at the vascular cell appears to be an important cause. For this reason, an attempt has been made to use sulfhydryl groups containing compounds to reverse nitrate tolerance in humans. Packer et al. showed partial restoration of NTG-mediated hemodynamic effect after the development of nitrate tolerance in a similar group of patients after the administration of NAC. May et al. reported complete reversal of tolerance to the effect of NTG on coronary blood flow after NAC administration. Ghio et al. demonstrated restoration of the attenuated venodilatory effect of NTG in the tolerant state in patients with ischemic heart disease with NAC. Levy et al. showed reversal of nitrate tolerance in patients with
N-acetylcysteine (NAC).

Ischemic heart disease after the administration of methionine, and two other preliminary studies reported partial reversal of NTG tolerance with the same agent in patients with chronic heart failure. In contrast to these data, Parker et al failed to demonstrate reversal of nitrate tolerance with NAC in patients with angina pectoris. Fung et al, who found in an animal experiment that incubation of nitrates with NAC accelerated the rate of denitrification of NTG but not of ISDN, speculated lack of NAC-ISDN interaction due to slow NAC-catalyzed production rate of S-nitrosothiol from ISDN. However, this speculation is not supported by the results of the present study, which demonstrate the presence of thiol-ISDN interaction in patients with heart failure. In addition, two recent studies demonstrating potentiation of the initial antischismic effect of ISDN with both NAC and captopril support the existence of thiol-ISDN interaction also in patients without heart failure.

The present study as well as the studies by Metelitsa et al and Boesgaard et al demonstrate potentiation of the initial ISDN effect before the development of nitrate tolerance. Failure to reverse tolerance to ISDN of NAC as shown by Parker et al but not to NTG as documented by other studies may suggest that in contrast to NTG, ISDN metabolism and interaction with NAC require normal levels of intracellular thiols and may not occur in the sulfhydryl-depleted state after chronic administration. Since NAC is more effective in increasing extracellular than intracellular sulfhydryl groups, other sulfhydryl donors such as oxothiazolidine, which is more effective than NAC in increasing intracellular sulfhydryl levels, may be more likely to reverse tolerance to ISDN.

Potential Limitations

Although the present study was performed in a randomized, crossover fashion, it is limited by its unblinded determinations of hemodynamic parameters. This limitation may be minimized by supportive changes in ANP, which was determined blindly. The failure to have a separate determination of the hemodynamic effect of NAC may be another potential limitation of the present study. However, lack of intrinsic vasodilator effect of NAC has been well demonstrated in previous studies in patients with ischemic heart disease and in patients with chronic CHF.

Although the immediate clinical applicability of the results of our study are limited, a better understanding of thiol-nitrates interaction may prove in the future to be therapeutically useful for potentiation of nitrates effect and prevention of nitrate tolerance.

Summary

This study demonstrates an augmentation of the hemodynamic and hormonal effects of oral ISDN after exogenous sulfhydryl group supplementation through the administration of intravenous NAC. Our findings therefore dispute previous suggestions for lack of interaction between NAC and ISDN due to slow NAC-catalyzed production of S-nitrosothiol from ISDN. The potentiation of the initial effect in contrast to the previously reported failure to reverse ISDN tolerance with NAC may suggest that NAC-ISDN interaction requires a normal level of intracellular sulfhydryl group and may not occur in intracellularly sulfhydryl-depleted state.

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References


