Enhancement of Endothelium-Dependent Vasodilation by Low-Dose Nitroglycerin in Patients With Congestive Heart Failure

Michael Schwarz, MD; Stuart D. Katz, MD; Laura Demopoulos, MD; Herbert Hirsch, MD; Jeannette L. Yuen, MD; Guillaume Jondeau, MD; Thierry H. LeJemtel, MD

Background Since organic nitroesters and endothelium-derived nitric oxide mediate vasodilation through a final common pathway, that is, by activation of soluble guanylate cyclase in vascular smooth muscle, nitroglycerin (NTG) could specifically enhance the endothelium-dependent vasodilatory response to acetylcholine (Ach) in patients with congestive heart failure (CHF) and endothelial cell dysfunction. Accordingly, the net effects of an intra-arterial infusion of NTG (10^-9 mol/L) on endothelium-dependent and endothelium-independent vasodilation were assessed in the forearm circulation of patients with CHF.

Methods and Results The forearm blood flow responses to intra-arterial administration of graded concentrations of Ach (10^-7 to 10^-5 mol/L) were determined by venous occlusion plethysmography (mL/min per 100 mL) in 18 patients with CHF and 5 age-matched normal subjects before and during intra-arterial infusion of NTG (10^-9 mol/L) for 20 minutes. In eight patients, the duration of the infusion of NTG (n=5) or vehicle control solution (n=3) was extended to 12 hours with measurement of the forearm blood flow responses to Ach at 20 minutes, 4 hours, and 12 hours. In five additional patients, forearm blood flow response to intra-arterial administration of two doses of phentolamine (0.05 and 0.5 mg) were determined before and during a 20-minute NTG infusion. Regional administration of NTG 10^-9 mol/L did not change resting forearm blood flow in either normal subjects or patients with CHF. Before administration of NTG 10^-9 mol/L, intra-arterial infusions of Ach 10^-7, 10^-6, and 10^-5 mol/L increased forearm blood flow to 14.7±6.2, 20.2±4.7, and 38.4±7.9 mL/min per 100 mL in normal subjects and to 4.1±0.8, 5.0±1.1, and 10.6±2.3 mL/min per 100 mL in patients with CHF. After administration of NTG 10^-9 mol/L for 20 minutes, the vasodilatory response to Ach significantly increased to 5.6±1.0, 6.9±1.6, and 17.7±3.4 mL/min per 100 mL in patients with CHF but did not change in normal subjects. The enhanced forearm blood flow responses to administration of Ach observed after 20 minutes of NTG administration in patients with CHF were sustained throughout a 12-hour NTG infusion. In contrast, regional administration of NTG did not change the vasodilatory responses to phentolamine.

Conclusions NTG, when administered intra-arterially for 20 minutes at a dose that does not affect resting forearm blood flow, significantly increased the vasodilatory response to intra-arterial administration of Ach in patients with CHF but not in normal subjects. The vasodilatory response to Ach was consistently enhanced by low-dose NTG throughout a 12-hour period. The vasodilating effects of organic nitroesters on the peripheral vasculature of patients with CHF may result in part from an interaction with the vascular endothelium. (Circulation. 1994;89:1609-1614.)

Key Words • blood flow • acetylcholine

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enhance vascular endothelium function by additive activation of soluble guanylate cyclase.\textsuperscript{12} Such a dose of NTG should specifically enhance endothelium-dependent vasodilation in patients with depressed basal endothelial production of nitric oxide but should have no detectable effect in subjects with normal endothelium function.

Accordingly, the present study was undertaken to assess the regional vascular responses to Ach, an endothelium-dependent vasodilator, and phentolamine, an endothelium-independent vasodilator, before and during intra-arterial administration of NTG (10\textsuperscript{-6} mol/L) in the forearm circulation of normal subjects and patients with CHF. As hemodynamic tolerance to the effects of intravenous NTG has been documented within a few hours of the onset of administration in patients with CHF,\textsuperscript{13,14} the regional vascular effects of NTG on endothelium-dependent vasodilation also were assessed throughout a 12-hour period.

**Methods**

**Patient Population**

Thirty-one patients with idiopathic dilated cardiomyopathy and CHF of at least 3 months’ duration and five normal subjects were studied. Subjects were entered into one of the three study protocols as described below. Eighteen patients (14 men and 4 women) and 5 normal subjects (3 men and 2 women) participated in protocol 1. The mean ages of patients with CHF and normal subjects were similar (55±10 versus 52±7 years, respectively, \(P=\)NS). Fifteen patients were in functional class III and three patients were in functional class II according to the criteria of the New York Heart Association. The left ventricular ejection fraction determined by radionuclide angiography averaged 19±7%. Medications included diuretics in 18 patients, digoxin in 15 patients, angiotensin-converting enzyme inhibitors in 13 patients, and long-acting nitrate preparations in 3 patients. The five subjects serving as normal controls underwent a physical examination, serum chemistries, and 12-lead ECG, which were all within normal limits.

Eight patients with CHF (six men and two women) participated in protocol 2. Five patients received intra-arterial administration of NTG and three received intra-arterial administration of vehicle control for 12 hours. The mean age of these patients was 60±8 years (range, 50 to 73). All patients were in functional class III. Left ventricular ejection fraction determined by radionuclide angiography averaged 21±6%. Medications included diuretics and digoxin in eight patients, angiotensin-converting enzyme inhibitors in seven patients, and long-acting nitrate preparations in three patients.

Five patients (three men, two women) participated in protocol 3. The mean age of these patients was 50±10 years. One patient was in functional class II and four were in functional class III. Left ventricular ejection fraction determined by radionuclide angiography averaged 25±4%. Medications included digoxin, diuretics, and angiotensin-converting enzyme inhibitors in all patients; one was receiving long-acting nitrates.

Cardiovascular medications were withheld for at least 24 hours before participation in the study. Patients with hypertension, diabetes mellitus, hypercholesterolemia (serum cholesterol >240 mg/dL), and coronary artery disease (as evidenced by coronary angiography or previously documented myocardial infarction) were excluded from the study. None of the study subjects were smokers. The study protocol was approved by the Ethical Review Board of the Albert Einstein College of Medicine. Written informed consent was obtained from all patients and normal subjects before participating in the study.

**Forearm Blood Flow Measurements**

Forearm blood flow was measured by venous occlusion plethysmography (mL/min per 100 mL of forearm volume) as previously described.\textsuperscript{15} A mercury-in-silastic strain gauge was placed at the widest part of the upper third of the forearm with the arm positioned comfortably above the level of the right atrium. The strain gauge was electrically coupled to a plethysmograph (Parks Electronics) calibrated to measure percent change in volume. The plethysmographic tracings of forearm blood flow were recorded on photographic paper for analysis (Electronics for Medicine, model VR-6). For each measurement, forearm venous blood flow return was occluded just above the elbow with a cuff rapidly inflated to 40 mm Hg (Hokanson Instruments, model E-20). Hand circulation was excluded from the blood flow measurement with a wrist cuff inflated to suprasystolic pressure 1 minute before and during each forearm blood flow measurement. The venous occluding cuff was inflated for 5 seconds at 15-second intervals; five blood flow recordings were averaged for each measurement. During each blood flow measurement, mean arterial pressure was recorded in the left arm at 15-second intervals by an automated cuff method (Critikon Dinamap Vital Signs Monitor, model 18465SX).

**Drug Administration**

All drugs were prepared fresh in 5% dextrose in water on the day of the study. For protocols 1 and 2, Ach, an endothelium-dependent vasodilating agent, was prepared to obtain regional blood concentrations of 10\textsuperscript{-7}, 10\textsuperscript{-6}, and 10\textsuperscript{-5} mol/L. Regional blood concentrations were estimated from measurements of baseline forearm blood flow. Each concentration was administered serially in the brachial artery as a 2-minute continuous infusion at 2-minute intervals. For protocol 3, phentolamine, an endothelium-independent vasodilator, was administered at two doses of 0.05 and 0.5 mg, respectively. Each dose was sequentially administered in the brachial artery as a 2-minute continuous infusion. For all protocols, NTG was administered to obtain final blood concentrations of 10\textsuperscript{-9} mol/L. This dose, which is equivalent to serum levels achieved during therapy with transdermal NTG preparations (10 mg per day),\textsuperscript{16} is one order of magnitude below the minimum dose of NTG that had been demonstrated previously to induce vasodilation in the forearm circulation of normal subjects.\textsuperscript{13} NTG 10\textsuperscript{-9} mol/L was administered in the brachial artery as a 20-minute continuous infusion to 18 patients and 5 normal subjects in protocol 1 and to 5 patients in protocol 3 and as a 12-hour continuous infusion in 5 patients in protocol 2.

**Study Protocols**

Subjects were studied in a resting supine position in the postabsorptive state in a quiet, temperature- and humidity-controlled room. A 20-gauge angiocath was inserted into the right brachial artery under local anesthesia (1% lidocaine) for intra-arterial drug administration. Thirty minutes after catheter placement, resting forearm blood flow was determined.

**Protocol 1: Effect of 20-Minute NTG Infusion on Endothelium-Dependent Vasodilation**

In 18 patients with CHF and 5 normal subjects, resting forearm blood flow and the forearm blood flow responses to administration of graded concentrations of Ach were determined before and at the end of a 20-minute infusion of NTG. Forearm blood flow was measured during the last minute of each infusion of Ach. Thirty minutes after completion of the NTG infusion, a vehicle control solution (5% dextrose in water) was administered into the brachial artery for 20 minutes in eight patients to determine the reproducibility of the vasodilatory response to Ach. Resting forearm blood flow and forearm blood flow responses to administration of graded...
concentrations of Ach infusions were determined before and during the 20-minute vehicle control infusion.

Protocol 2: Effect of 12-Hour NTG Infusion on Endothelium-Dependent Vasodilation

Eight patients with CHF were randomly assigned to receive either NTG 10^{-9} mol/L (n=5) or 5% dextrose in water (vehicle control, n=3) administered in the brachial artery as a 12-hour continuous infusion. The forearm blood flow responses to administration of graded concentrations of Ach were determined before administration of NTG or vehicle control and after 20 minutes, 4 hours, and 12 hours of the infusion.

Protocol 3: Effect of 20-Minute NTG Infusion on Endothelium-Independent Vasodilation

In five additional patients with CHF, resting forearm blood flow and the forearm blood flow responses to administration of two doses of phentolamine were determined before and at the end of a 20-minute infusion of NTG 10^{-9} mol/L. Forearm blood flow was measured during the last minute of each administration of phentolamine.

Statistical Analysis

All values are reported as mean±SE. Within-group comparisons (protocols 1 and 3) of the regional forearm blood flow responses to administration of Ach and phentolamine before and during administration of NTG and vehicle control were analyzed with a two-factor, repeated-measures ANOVA model. Between-group comparisons (protocol 1) were analyzed with a split-plot ANOVA model. In protocol 2, forearm blood flow responses for Ach after 20 minutes, 4 hours, and 12 hours of NTG infusion were analyzed with one-way, repeated-measures ANOVA. A P value of <.05 was considered statistically significant.

Results

Protocol 1: Effect of 20-Minute NTG Infusion on Endothelium-Dependent Vasodilation

In five normal subjects, administration of NTG 10^{-9} mol/L did not change resting forearm blood flow (4.6±0.3 versus 4.4±0.5 mL/min per 100 mL, P=NS; Fig 1, panel A). Before administration of NTG 10^{-9} mol/L, intra-arterial infusions of Ach 10^{-7}, 10^{-6}, and 10^{-5} mol/L increased forearm blood flow to 14.7±6.2, 20.2±4.7, and 38.4±7.9 mL/min per 100 mL, respectively. During administration of NTG 10^{-9} mol/L, the forearm blood flow responses to Ach did not change (Fig 1, panel A). In 18 patients with CHF, administration of NTG 10^{-9} mol/L did not change resting forearm blood flow (2.8±0.3 versus 3.0±0.4 mL/min per 100 mL, P=NS; Fig 1, panel B). Before administration of NTG 10^{-9} mol/L, intra-arterial infusions of Ach 10^{-7}, 10^{-6}, and 10^{-5} mol/L increased forearm blood flow to 4.1±0.8, 5.0±1.1, and 10.6±2.3 mL/min per 100 mL, respectively. During administration of NTG 10^{-9} mol/L, intra-arterial infusions of Ach increased forearm blood flow to 5.6±1.0, 6.9±1.6, and 17.7±3.4 mL/min per 100 mL, respectively. The increases in forearm blood flow in response to intra-arterial infusions of Ach 10^{-9} mol/L and 10^{-5} mol/L were significantly greater during administration of NTG when compared with those observed before NTG administration (both P<.05; Fig 1, panel B). The enhanced response to Ach during administration of NTG was greatest at the 10^{-5} mol/L dose when the forearm blood flow increased by 70% (from 10.6 to 17.7 mL/min per 100 mL, P<.01). A 20-minute infusion of a vehicle control solution did not change the forearm blood flow response to regional administration of graded concentrations of Ach in eight patients with CHF (Table 1).

When compared with resting forearm blood flow, forearm blood flow responses to administration of Ach were significantly increased in normal subjects and in patients with CHF both before and at the end of NTG infusion (P<.05 for within-group comparisons of all doses of Ach). When compared with normal subjects, the forearm blood flow responses to administration of Ach were significantly reduced in patients with CHF both before and at the end of NTG infusion (P<.05 for between-group comparisons for all doses of Ach). Heart rate and mean arterial pressure did not change in patients with CHF or normal subjects during drug infusions.

Protocol 2: Effect of 12-Hour NTG Infusion on Endothelium-Dependent Vasodilation

In five patients with CHF, intra-arterial infusions of Ach 10^{-7}, 10^{-6}, and 10^{-5} mol/L increased forearm blood flow to 2.3±0.4, 3.6±0.7, and 21.6±6.0 mL/min per 100
mL, respectively. At 20 minutes during continuous NTG infusion, the forearm blood flow responses to intra-arterial administration of Ach $10^{-7}$, $10^{-6}$, and $10^{-5}$ mol/L increased to 3.5±0.4, 4.4±0.5, and 25.7±7.2 mL/min per 100 mL, respectively. The increases in forearm blood flow in response to repeated intra-arterial administration of Ach at 20 minutes, 4 hours, and at the end of a 12-hour infusion of NTG $10^{-5}$ mol/L were similar (Fig 2). In three patients with CHF, forearm blood flow responses to intra-arterial administration of Ach did not change during a 12-hour infusion of vehicle control solution (Table 2).

**Protocol 3: Effects of 20-Minute NTG Infusion on Endothelium-Independent Vasodilation**

In five additional patients with CHF, forearm blood flow responses to administration of 0.05 and 0.5 mg of phentolamine were similar before and at the end of a 20-minute infusion of NTG $10^{-4}$ mol/L (Table 3).

**Discussion**

The current data confirm that the peripheral vasodilatory response to intra-arterial administration of an endothelium-dependent vasodilator, Ach, is decreased in patients with CHF when compared with that of age-matched normal subjects. Moreover, the data indicated that NTG at a dose ($10^{-9}$ mol/L) that, by itself, did not exert any detectable hemodynamic effect, substantially enhanced the vasodilatory response to Ach, an endothelium-dependent vasodilator, in patients with CHF but did not in normal subjects. The enhancement of the vasodilatory response to Ach by low-dose NTG was sustained throughout the 12-hour period. In contrast, administration of NTG did not change the forearm blood flow responses to phentolamine, an endothelium-independent vasodilator.

These findings argue in favor of a synergistic relation between NTG and endothelium-derived nitric oxide in patients with CHF. Of interest, previous animal studies have reported inhibitory interactions between endothelium-derived nitric oxide and NTG. In isolated vascular segments, mechanical removal of the vascular endothelium or inhibition of endothelium-derived nitric oxide production increases the vasodilatory response to NTG. Similarly, in Wistar rats, systemic inhibition of nitric oxide synthase acutely augments the hypotensive effects of systemic NTG administration. The inhibitory effect of the vascular endothelium on the vasodilating properties of organic nitroesters has been attributed to the development of cross-tolerance between exogenous and endogenous sources of nitric oxide. Cross-tolerance among nitrovasodilators is characterized by a dose-dependent diminished stimulation of soluble guanylate cyclase activity in vascular smooth muscle. Of note, the inhibitory effects of the vascular endothelium could be related to other endothelium-derived vasoactive substances such as superoxide anion. Moreover, some investigators have failed to observe a cross-tolerance between organic nitroesters and endothelium-derived nitric oxide. The apparent disparity between our findings and previous experimen-

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**Table 1.** Forearm Blood Flow Responses to Intra-arterial Administration of Graded Concentrations of Acetylcholine Before and During a 20-Minute Infusion of Vehicle Control Solution (5% Dextrose in Water) in Patients With Congestive Heart Failure

<table>
<thead>
<tr>
<th>Forearm Blood Flow, mL/min per 100 mL</th>
<th>Baseline Ach, $10^{-7}$ mol/L</th>
<th>Ach, $10^{-6}$ mol/L</th>
<th>Ach, $10^{-5}$ mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before vehicle control</td>
<td>2.6±0.4</td>
<td>2.7±0.5</td>
<td>3.3±0.8</td>
</tr>
<tr>
<td>After vehicle control</td>
<td>2.6±0.6</td>
<td>2.7±0.7</td>
<td>3.0±0.7</td>
</tr>
</tbody>
</table>

Ach indicates acetylcholine. n=8 patients.

**Table 2.** Forearm Blood Flow Responses to Intra-arterial Administration of Graded Concentrations of Acetylcholine Before and After 20 Minutes, 4 Hours, and 12 Hours of Continuous Administration of Vehicle Control Solution (5% Dextrose in Water) in Patients With Congestive Heart Failure

<table>
<thead>
<tr>
<th>Forearm Blood Flow, mL/min per 100 mL</th>
<th>Ach, $10^{-7}$ mol/L</th>
<th>Ach, $10^{-6}$ mol/L</th>
<th>Ach, $10^{-5}$ mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>3.6±0.5</td>
<td>9.5±1.5</td>
<td>21.2±1.8</td>
</tr>
<tr>
<td>20 Minutes</td>
<td>3.6±0.4</td>
<td>10.8±1.1</td>
<td>20.1±1.0</td>
</tr>
<tr>
<td>4 Hours</td>
<td>3.5±0.3</td>
<td>10.7±1.0</td>
<td>19.7±1.1</td>
</tr>
<tr>
<td>12 Hours</td>
<td>4.0±0.6</td>
<td>9.2±1.5</td>
<td>19.2±2.7</td>
</tr>
</tbody>
</table>

Ach indicates acetylcholine. n=3 patients.
tal data may be related to baseline abnormalities of vascular endothelial function in CHF. Cross-tolerance may not develop in patients with CHF who have chronically decreased basal levels of endothelium-derived nitric oxide. The lack of an increase in endothelium-dependent vasodilation during intra-arterial administration of NTG in normal subjects supports this hypothesis.

The mechanisms that mediate impairment of vascular endothelial cell function in patients with CHF are not fully characterized. Chronic reduction of shear stress on the vascular endothelial cells, increased degradation of nitric oxide, and reduced vascular smooth muscle response to soluble guanylate cyclase stimulation may all contribute to impairment in endothelium-dependent vasodilation. Increased levels of cGMP in the vascular smooth muscle cells may be responsible for the increased vasodilatory effects of Ach after administration of low-dose NTG. NTG, in the concentration used in this study (10^{-9} mol/L), has been demonstrated to increase cGMP levels and mediate vasorelaxation in vascular smooth muscle in isolated blood vessels. In rabbit aorta, concomitant administration of the nitric oxide donor SIN-1 and endothelium-derived nitric oxide has been reported to act additively to increase the cGMP content of vascular smooth muscle, whereas additive effects on vasorelaxation were not observed. In the present study, administration of NTG 10^{-9} mol/L increased the vasodilatory response to Ach in patients with CHF without exerting any detectable effects on resting forearm blood flow. The increase in intracellular cGMP produced by 10^{-9} mol/L of NTG probably is not sufficient to promote vascular smooth muscle relaxation in the basal state. However, when cGMP production has been activated by Ach, the increase in cGMP produced by 10^{-9} mol/L of NTG then may result in further relaxation of the vascular smooth muscle. This additive effect of low-dose NTG may have been more readily evident in patients with CHF in whom basal stimulation of soluble guanylate cyclase is reduced when compared with that of normal subjects. The absence of change in the blood flow responses to phenolamine, an endothelium-independent vasodilator, during NTG infusion further supports the specific role of cGMP in mediating the interaction between NTG 10^{-9} mol/L and Ach.

Early tolerance to the vasodilating effects of organic nitroesters has been documented in patients with CHF. The mechanisms that contribute to the attenuation of the vasodilating effects of organic nitroesters include (1) decreased biotransformation of organic nitroesters to nitric oxide, (2) decreased sensitivity of soluble guanylate cyclase to nitric oxide, and (3) reflex neurohormonal activation with secondary changes in peripheral vasomotor tone and intravascular volume. The absence of tolerance to the effects of NTG on endothelium-dependent vasodilation in the current study may be attributed to several factors. First, the low dose of NTG administered in this study may have reduced the likelihood of the development of tolerance. In isolated blood vessels, the development of tolerance after 2 hours of exposure to NTG 10^{-10} mol/L was substantially less than that after 2 hours of NTG 10^{-7} mol/L. In clinical studies, the development of tolerance to the hemodynamic effects of organic nitroesters also may be, in part, dose related. Second, regional administration of NTG 10^{-9} mol/L did not exert systemic hemodynamic effects, and thereby acute neurohormonal activation is not likely to have occurred. It is possible that the 12-hour period of observation in this study was too brief to observe the development of tolerance. However, substantial attenuation of the acute hemodynamic effects of organic nitroesters has been described within 4 to 8 hours of continuous intravenous administration in previous studies of nitrate tolerance.

The mechanisms that mediate the vascular effects produced by systemic administration of NTG at therapeutic doses are likely to be more complex than that reported in the present study, which involved regional administration of NTG at 10^{-9} mol/L. Nevertheless, our results suggest that the hemodynamic effects of NTG may, in part, result from an interaction with the vascular endothelium. Endothelium-dependent vasodilation, which is depressed in the basal state in patients with CHF, also is likely to be abnormal during physiological states of increased shear stress, such as during intense exercise. Whether systemic administration of NTG at therapeutic doses does enhance flow-mediated endothelium-dependent vasodilation during exercise in patients with CHF remains to be studied.

TABLE 3. Forearm Blood Flow Responses to Intra-arterial Administration of Two Doses of Phentolamine Before and During a 20-Minute Infusion of Nitroglycerin in Five Patients With Congestive Heart Failure

<table>
<thead>
<tr>
<th>Forearm Blood Flow, mL/min per 100 mL</th>
<th>Baseline</th>
<th>Phenolamine, 0.05 mg</th>
<th>Phenolamine, 0.5 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before nitroglycerin, 10^{-9} mol/L</td>
<td>2.1±0.5</td>
<td>4.1±1.2</td>
<td>5.1±1.6</td>
</tr>
<tr>
<td>At end of nitroglycerin, 10^{-9} mol/L</td>
<td>2.0±0.5</td>
<td>4.3±1.2</td>
<td>5.2±1.9</td>
</tr>
</tbody>
</table>

References

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