Nitroglycerin Withdrawal Increases Endothelium-Dependent Vasomotor Response to Acetylcholine

Eduardo R. Azevedo, MD, Anne M. Schofield, BScN, RN, Susan Kelly, BScN, RN, John D. Parker, MD, FACC

Toronto, Ontario, Canada

OBJECTIVES

We sought to determine whether nitroglycerin (NTG) withdrawal contributes to worsening of endothelial dysfunction and development of the rebound phenomenon during intermittent transdermal NTG therapy.

BACKGROUND

Intermittent transdermal NTG therapy is recommended to avoid the development of tolerance. However, this regimen may precipitate worsening angina in the NTG-free interval.

METHODS

Twenty patients were randomized to intermittent transdermal NTG (0.6 mg/h; NTG group) or no treatment (control group) five days before angiography. The risk factors for endothelial dysfunction were similar in both groups. After diagnostic angiography, the patients underwent quantitative angiography before and after intracoronary acetylcholine (ACh), \(10^{-4}\) mol/liter. Immediately after the morning study, the patch was removed from the NTG group, and 3 h later, the ACh infusion was repeated in both groups. All patients had mild to moderate coronary artery disease (CAD).

RESULTS

The diameter of the left anterior descending coronary artery at baseline was 2.0 ± 0.1 mm in the control group and 2.6 ± 0.1 mm in the NTG group (\(p < 0.05\)). Acetylcholine caused mild vasoconstriction in the control group in the morning and afternoon (2.7 ± 5.3% and 2.4 ± 3.9%, respectively; \(p = NS\)). The NTG group demonstrated mild vasoconstriction to ACh in the morning (3.2 ± 2.8%; \(p = NS\) vs. control group). After patch removal, there was a significant increase in the magnitude of vasoconstriction in the NTG group (11.6 ± 3.9%, \(p = 0.04\) vs. morning constriction).

CONCLUSIONS

These results confirm that NTG withdrawal increases the coronary vasomotor response to ACh in patients with mild CAD and suggests that the rebound phenomena may be secondary to the development of endothelial dysfunction after discontinuation of NTG therapy. (J Am Coll Cardiol 2001;37:505–9) © 2001 by the American College of Cardiology

An intermittent dosing regimen with a daily drug-free interval is required to avoid the development of tolerance to transdermal nitroglycerin (NTG) (1–3). A potential complication of this drug-free period is the development of rebound myocardial ischemia during the nitrate-free interval (2,4,5). Indeed, withdrawal after exposure to NTG has been associated with angina pectoris, myocardial infarction and even sudden death (6,7).

Although the cause of nitrate tolerance remains unclear, several different mechanisms have been proposed (8). Recent evidence suggests that nitrate exposure is associated with increased vascular sensitivity to vasoconstrictors, a phenomenon which may be secondary to increased vascular production of free radicals (9) or endothelin, or both (10). In a previous study, we demonstrated that continuous therapy with transdermal NTG was associated with the development of endothelial dysfunction, as assessed by the coronary vasomotor response to acetylcholine (ACh) (11). In this study, withdrawal of NTG therapy for 3 h was associated with even greater evidence of endothelial dysfunction. The cause of NTG-induced endothelial dysfunction remains unknown; however, it may be associated with specific changes in vascular biochemistry, such as increased superoxide anion production or changes in vascular production of endothelin (9,10).

In the present study, we examined the impact of intermittent transdermal NTG therapy and the early nitrate-free interval on coronary endothelial function. We hypothesized that 1) endothelial dysfunction would not develop during the initial 12 h of transdermal NTG therapy; and 2) endothelial dysfunction, as manifested by an increased sensitivity to ACh-induced coronary vasoconstriction, would be observed during the period after removal of transdermal NTG.

METHODS

Study group. Patients referred for diagnostic coronary angiography were eligible to participate in this study. Clinical exclusion criteria included previous coronary artery bypass graft surgery or percutaneous transluminal coronary angioplasty; unstable angina or myocardial infarction within 30 days of randomization; second- or third-degree atrioventricular block; and clinically significant renal or hepatic disease. At the time of catheterization, those with evidence
of left main coronary artery (LMCA) atherosclerosis or significant coronary artery disease (CAD; stenosis ≥60%) involving the left anterior descending coronary artery (LAD) or circumflex coronary artery (Cx) were excluded from the study. Of the 48 patients enrolled in this study, 21 were excluded because of significant CAD involving the LMCA, LAD or Cx. Five patients with angiographically normal coronary arteries were also excluded. In two patients, the study was interrupted because of severe bradycardia or second-degree atrioventricular block, and in one patient, the protocol was stopped because of technical difficulties. All complications were treated promptly, and no patient required temporary pacing or atropine.

**Study protocol.** The patients were randomly assigned to receive intermittent transdermal NTG (Ciba-Geigy, Mississauga, Ontario), 0.6 mg/h, or no treatment (we could not obtain placebo NTG patches), in an investigator-blinded fashion. All patients taking long-acting nitrates were asked to stop this medication at the time of randomization. Other vasodilators were discontinued 48 h before the study. Patients were allowed to use sublingual NTG, as required for relief of angina, but no study was carried out during the course of the study. A significant increase in blood pressure either group in response to the infusion of ACh. In the non-NTG group, there was no change in blood pressure during the morning, noon angiograms. Furthermore, the differences between the groups in the overall response to ACh during the morning, noon angiograms. Statistical analysis. The patients' baseline characteristics were compared using the unpaired t test for continuous variables. The Fischer exact test was used to compare binary variables. Changes in the mean lumen diameter, from control values to each dose of ACh, were compared using multivariate analysis of variance within a general linear model procedure, with appropriate contrast statements (SAS version 6.11, SAS Institute Inc., Cary, North Carolina). This model allowed for analysis of intergroup changes in the responses to ACh for both the morning and afternoon angiograms. Furthermore, the differences between the groups in the overall response to ACh during the morning, versus the afternoon, could also be compared. Finally, the procedure also allowed for univariate comparisons at baseline and all subsequent time points between groups. A two-sided p value <0.05 was considered significant.

**RESULTS**

**Patient characteristics.** Ten patients were randomized to the non-NTG arm (nine men, 59 ± 4 years old) and 10 patients to NTG therapy (seven men, age 58 ± 3 years old). The groups were well matched in terms of baseline characteristics and risk factors for endothelial dysfunction (Table 1). All patients had CAD, as manifested by intimal irregularities or mild to moderate obstructive disease.

**Hemodynamic responses.** Heart rate did not change in either group in response to the infusion of ACh. In the non-NTG group, there was no change in blood pressure during the course of the study. A significant increase in
systolic (from 133 ± 6 to 146 ± 7 mm Hg, control AM vs. control PM; p < 0.05) and diastolic blood pressure (from 69 ± 3 to 74 ± 3 mm Hg, control AM vs. control PM; p < 0.05) was observed after removal of the patch in the NTG group.

**Responses of coronary arteries.** At baseline, the mean diameter of the LAD in the non-NTG group was 2.02 ± 0.09 mm, which was significantly smaller than the 2.64 ± 0.10 mm observed in the NTG group (p < 0.05).

In the non-NTG group, the peak dose of ACh caused a very small reduction in the mean lumen diameter, from 2.02 ± 0.09 to 1.97 ± 0.14 mm (p = 0.11), which represents a mean vasoconstriction of only 2.7 ± 5.3% (Fig. 1). In the afternoon, the peak dose of ACh had an almost identical effect on the non-NTG group and caused a change in the mean lumen diameter of the LAD from 2.02 ± 0.10 to 1.98 ± 0.15 mm (p = 0.13), which also represents a nonsignificant vasoconstriction of 2.4 ± 3.9% (Fig. 1). There was absolutely no difference between the morning and afternoon responses to ACh (p = 0.93). In the NTG group, during the morning part of the study, while the transdermal NTG patch was still in place, there was only a very mild effect of the peak dose of ACh on the coronary vasomotor response. The mean lumen diameter of the LAD went from 2.64 ± 0.10 to 2.56 ± 0.12 mm (p = 0.67), which represents a mean vasoconstriction of 3.2 ± 2.8% (Fig. 1). During the afternoon protocol, 3 h after removal of the NTG patch, the mean diameter of the LAD was reduced to 2.17 ± 0.08 at baseline (p < 0.05 vs. morning control, NTG group). The intracoronary infusion of ACh caused a further reduction in the LAD diameter to 1.93 ± 0.12 mm (p < 0.05 vs. afternoon control, NTG group), which represents a vasoconstriction of 11.6 ± 3.9% (p = 0.04 vs. morning constriction, NTG group) (Fig. 1). Multivariate analysis of variance revealed that there was a highly significant difference between the responses of the NTG group and those of the non-NTG treatment group (p < 0.001). The reduction in the LAD diameter after patch removal, as well as the significant vasoconstrictive response to ACh observed in the afternoon, are illustrated in Figure 2.

**DISCUSSION**

The development of tolerance to continuous transdermal NTG therapy occurs rapidly after the initiation of therapy and carries important clinical implications. The only effective method of preventing tolerance to NTG has been the use of dosing schedules that provide low or absent plasma NTG levels for a portion of the day. A number of studies have determined that intermittent therapy with transdermal NTG is effective in the prevention of tolerance, and this regimen is approved and indicated for the treatment of angina. However, there are potential problems associated with intermittent NTG therapy. A patient receiving monotherapy with nitrates will have no coverage during the nitrate-free period and may require combined therapy with a beta-blocker or calcium channel blocker. In addition, there is also concern that anginal symptoms may be exacerbated during the nitrate-free period, because of a phenomenon known as “rebound ischemia” (6). Although the cause of rebound ischemia is uncertain, there is evidence to suggest that this is a clinically relevant problem.

**Rebound ischemia.** For nearly 50 years, several studies have reported what was termed the “withdrawal hazards” of
NTG. In most cases, this was manifested as worsening angina, myocardial infarction or sudden death among workers of the explosives industry after they had left the plant for two or three days (6,7). Given the nature of NTG exposure in these reports, it is difficult to extrapolate these observations to the clinical use of this compound. In more recent investigations of intermittent NTG therapy, an increase in angina and myocardial ischemia during the nitrate-free interval was seen (2,4,14). In one multicenter, randomized controlled trial evaluating the efficacy of intermittent transdermal NTG in patients with stable angina, 9 of 138 patients receiving active treatment fulfilled the defined criteria for a significant increase in rest angina after patch removal. None of the 68 patients in the control group developed rest angina during the patch-off interval (2). In another placebo-controlled, crossover design study comparing the effects of continuous versus intermittent NTG therapy in patients with stable angina, a higher incidence of anginal attacks was seen during the NTG-free interval (4). Similar clinical results were documented more recently by Pepine et al. (14). Although these reports are a cause of concern, no such effects were reported in other studies (15–17), including a large-scale trial of intermittent NTG therapy (3).

Zero-hour effect. Intermittent transdermal NTG therapy has also been shown to have adverse effects on exercise performance during the nitrate-free interval. In patients with stable angina, intermittent transdermal NTG is associated with a decrease in the angina threshold for 4 to 6 h after discontinuation of therapy (5). In a similar study by Pepine et al., an increase in the frequency of angina during the patch-off interval was documented by patients, and this subjective finding was supported by a corresponding trend toward an increase in ambulatory electrocardiographic evidence of ischemia in this same period (14). DeMots and Glasser (2) demonstrated that 12 h after the removal of the transdermal NTG or placebo patch, the placebo group was able to exercise longer than the group receiving active therapy—a phenomenon that was called the “zero hour effect.” Similar results were reported in another large trial of intermittent NTG therapy (3).

Nitroglycerin and endothelial dysfunction. In a recent study by our group, we documented an abnormal coronary vasomotor response to the endothelium-dependent vasodilator ACh in patients undergoing continuous therapy with transdermal NTG (11). The abnormal vasoconstriction observed was accentuated after discontinuation of therapy. We hypothesized that these results were secondary to the development of endothelial dysfunction caused by biochemical changes in the coronary vasculature that occurred during NTG therapy. In the current study, we used a similar protocol to test the coronary vasomotor responses to ACh during intermittent therapy with transdermal NTG and after patch removal. This study documents that intermittent NTG therapy is not associated with worsening of endothelial dysfunction during the period of transdermal NTG exposure. Withdrawal of NTG, however, was associated with aggravating endothelial dysfunction. Patients receiving intermittent transdermal NTG demonstrated a significant increase in the magnitude of vasoconstriction 3 h after patch removal, an effect that was not seen in the control group. This interval of 3 h was chosen on the basis of the results of studies that documented a decline in NTG plasma concentrations to undetectable levels within 1 h of patch removal (18,19). This increase in the severity of vasoconstriction after NTG withdrawal was five times larger in the active therapy arm than in the control group (Fig. 1). There was no evidence of tolerance in the NTG arm, given the fact that in this group, the mean LAD diameter at baseline was significantly larger than that in the control group. The

Figure 2. Coronary angiograms from patients in the non-NTG group (panel A) and NTG group (panel B). The angiograms were obtained at the time of the morning control solution of D5W (Control AM), during 10^−4 mol/liter of ACh in the morning (ACh−4 AM), during the afternoon control solution of D5W (Control PM) and during 10^−4 mol/liter of ACh in the afternoon (ACh−4 PM). The white arrow identifies the LAD.
mechanism underlying this response is not clear. We hypothesize that during NTG therapy, biochemical changes develop that make the coronary vasculature more sensitive to vasoconstrictors (9,10,20). During the period of NTG exposure, these biochemical changes develop, but are balanced by the dilating effect of the nitrate. After withdrawal of NTG therapy, these biochemical changes persist, but are no longer opposed by the vasodilating properties of the nitrate. The resulting imbalance between dilator and constrictor systems is manifested as a greater sensitivity to the vasoconstrictor actions of ACh.

Study limitations. It is important to consider some limitations of this study. Differences in baseline characteristics can potentially influence our results. A crossover design would not be possible given the need for repeat coronary angiography. We carefully monitored all risk factors for endothelial dysfunction, because endothelium-dependent responses to ACh have been related to risk factors (21). There were no significant differences between the two groups for any of the baseline characteristics commonly reported in previous studies that evaluated the coronary vasomotor response to ACh (11,22).

Clinical relevance. We believe these findings have clinical significance. Although the incidence of clinically significant episodes of ischemia after transdermal NTG therapy is relatively low (it was seen in only 6.5% of the patients in the report by DeMots and Glasser [2]), physicians should be aware of this possibility in patients on transdermal therapy. In a recent, large, retrospective study, the use of long-term nitrate therapy appeared to be harmful in a group of patients with CAD. These analyses raise concern about the potential adverse effects of long-acting nitrate therapy in chronic CAD (23). Furthermore, the possibility of rebound ischemia suggests that intermittent transdermal therapy should be used with caution in patients with variable threshold angina or recent unstable ischemic episodes.

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Reprint requests and correspondence: Dr. John D. Parker, Mount Sinai Hospital, 600 University Avenue, Suite 1609, Toronto, Ontario M5G-1X5. E-mail: jdp@inforam.net.

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