Myocardial Ischemia

The Effect of Supplemental L-Arginine on Tolerance Development During Continuous Transdermal Nitroglycerin Therapy

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OBJECTIVES

This study was designed to assess the effect of oral L-arginine on the development of tolerance during continuous transdermal nitroglycerin (TD-GTN) therapy.

BACKGROUND

Continuous TD-GTN therapy leads to complete tolerance within 24 to 48 h. The mechanism(s) responsible for nitrate tolerance are unclear, but there is increasing evidence that nitroglycerin (GTN) leads to superoxide anion production. The trigger for this is unknown, but there is evidence that GTN alters nitric oxide synthase (NOS) function and also leads to reduced L-arginine availability at its site of action with NOS.

METHODS

Fourteen patients with stable angina pectoris and reproducible treadmill walking time (TWT) until the onset of moderate angina were studied in a placebo-controlled, crossover study. Transdermal GTN (0.4 mg/h) was applied daily for two periods of 5 to 10 days with the patch left in place for 24 h each day. Capsules containing L-arginine (700 mg) or placebo were administered four times daily during a period of TD-GTN therapy. Treadmill walking time was determined before and 4 h after study capsules on day 1 before TD-GTN to assess the effect of L-arginine on exercise performance. On the last day, TWT was determined at 0 h (24 h after TD-GTN and 9 h after study capsule) and 4 h after TD-GTN reapplication and study capsule. After a 5 to 10 day washout period, the study was repeated with the opposite study capsule.

RESULTS

Treadmill walking time until the onset of moderate angina was not influenced by the short-term administration of L-arginine. During continuous TD-GTN, the administration of L-arginine increased TWT 4 h and 24 h after patch application. This was significantly greater than TWT during administration of placebo capsules (p < 0.05).

CONCLUSIONS

The administration of L-arginine modified or prevented the development of nitrate tolerance during continuous TD-GTN therapy. (J Am Coll Cardiol 2002;39:1199–203) © 2002 by the American College of Cardiology Foundation

The organic nitrates are effective in the management of patients with angina pectoris and are considered by many to be first-line therapy for this disorder (1). However, the fact that tolerance develops rapidly during continuous therapy with nitrates limits their usefulness (2,3). Intermittent dosing, which provides a period of nitrate washout, has been shown to provide antianginal effects for 12 to 14 h, but this is inadequate in many patients (4,5). If it were possible to prevent or modify tolerance, the organic nitrates would be a much better treatment option.

The mechanism(s) responsible for nitrate tolerance are not completely understood. Hypotheses to explain this phenomenon include: decreased bioconversion of nitrate to nitric oxide (NO) due to sulphhydryl group depletion (6); neurohormonal activation with release of vasoactive agents (7,8); plasma volume expansion resulting in increased preload (9,10); decreased cyclic guanosine monophosphate production secondary to downregulation of guanylyl cyclase (11); increased breakdown of cyclic guanosine monophosphate due to increased phosphodiesterase activity (12); and superoxide anion production resulting in NO depletion (13). Attempts to prevent or reverse nitrate tolerance with sulphhydryl donors, angiotensin-converting enzyme inhibitors, angiotensin receptor blocking agents, hydralazine, diuretics and antioxidants have produced either negative or inconsistent results (1). As a result none of these interventions has been adopted into widespread clinical use.

In vitro studies have demonstrated an increase in superoxide anion production by endothelial cells exposed to glyceryl trinitrate (GTN) (14). The source of this abnormal superoxide anion production remains controversial, although both membrane-bound oxidases and nitric oxide synthase (NOS) itself have been implicated (15–17). The trigger of this increase in superoxide production in response to therapy with GTN remains unclear, although there is considerable evidence that depletion of arginine is involved (14,17). It has been hypothesized that intracellular depletion of L-arginine leads to an uncoupling of NOS because
of decreased substrate availability (14,17). This uncoupling, in turn, leads to an increase in the bioavailability of superoxide anion. This hypothesis suggests that arginine supplementation during sustained GTN therapy might prevent the development of nitrate tolerance. Continuous transdermal GTN (TD-GTN) induces tolerance within 24 h of application (2). The present study was designed to test the hypothesis that the supplemental administration of L-arginine during continuous TD-GTN therapy would prevent or modify tolerance in patients with stable angina pectoris.

METHODS

This was a single-center study in patients with stable angina pectoris. The protocol was approved by the Ethics Committee of the Queen’s University Affiliated Hospitals, and each patient provided informed, written consent.

To be eligible for this study, patients had to have documented coronary artery disease confirmed by evidence of a previous myocardial infarction, significant coronary angiography abnormalities, a myocardial perfusion scan showing reversible perfusion defects or a positive dobutamine or stress echocardiographic study. On initial assessment, patients had to develop moderate angina (P2) during treadmill exercise testing between 2 and 8 min using the standard Bruce protocol. The end point (P2) was defined as the point at which patients would normally stop activity and possibly take sublingual GTN for relief of pain. The treadmill exercise time to P2 had to be reproducible with two consecutive exercise tests carried out on different days being within 15% of one another.

Exclusion criteria included myocardial infarction, unstable angina, a revascularization procedure within two months, congestive heart failure, abnormal renal or liver function tests, hemoglobin below 10 gm/l, systolic blood pressure (BP) below 100 mm Hg or greater than 170 mm Hg, or the use of digoxin, angiotensin-converting enzyme inhibitors, angiotensin receptor blocking agents, amiodipine, carvedilol or vitamin C or E. During the study, patients were permitted to take beta adrenoreceptor blocking agents, calcium channel antagonists (other than amlodipine). The dose was kept constant throughout the study period, and on study days these medications were taken after the final exercise test. No long-acting nitrates were permitted, but patients could use sublingual GTN to treat episodes of angina. Patients were asked to avoid using sublingual GTN for 2 h before any exercise test. If it was necessary for the patient to take this medication, exercise testing was scheduled for another day.

This was a randomized, double-blind, placebo-controlled, crossover study comparing the effects of L-arginine capsules given four times daily and matching placebo during continuous TD-GTN application. After fulfilling the entry criteria and having none of the exclusion criteria, the definitive investigation was carried out. On the initial study day, treadmill exercise testing was carried out in the fasting state without taking any medications. The time to the development of moderate angina (P2) was determined. After this, the study capsule (either 700 mg L-arginine or placebo) was taken. Three to four hours later, a second exercise test was carried out to the same end point. Transdermal nitroglycerin (0.4 mg/h) was then applied and left in place until the following morning. For the next 5 to 10 days, the patients removed the TD-GTN each morning and replaced it with a new one. During this period, patients took the study capsules four times daily, with the last capsule taken at approximately 11 PM. On the final study day, the patients returned to the laboratory with the previous TD-GTN patch in place and without taking the morning study capsule. Exercise testing was carried out to the defined end point. A new TD-GTN patch was then applied, the study capsule was taken, and exercise testing was carried out after 3 to 4 h.

After the first study phase, there was a washout period of at least five days during which no study capsules, TD-GTN patches or long-acting oral nitrates were employed. After this washout period, the study was repeated in an identical fashion with continuous TD-GTN and the alternate study capsule administered.

The protocol design allowed us to determine whether L-arginine alone had an effect on exercise performance and whether the administration of L-arginine modified the development of tolerance during continuous TD-GTN therapy.

Statistical methods. The Wilk-Shapiro test was applied to test all data at all time points for normality (SAS release 6.11, SAS Institute Inc., Cary, North Carolina). The method of Hills and Armitage for analysis of a two-period crossover trial design was used to determine whether there was a treatment effect (L-arginine vs. placebo) on treadmill walking times (TWTs), heart rate and systolic BP (18). The same method was applied to examine the data for an effect of L-arginine alone on TWT. The analyses examined the data for the presence of an interaction between the two treatment arms. Such an interaction may be observed if one double-blind treatment period has significant effects on the subsequent treatment period. The analyses also examined for the presence of a period effect. A period effect is found when the observed result appears to be dependent on the order of treatment.
RESULTS

Fourteen patients who fulfilled the inclusion criteria and had none of the exclusion criteria completed this investigation. On the first study day of each of the two treatment phases (Table 1, Fig. 1), there was no difference in exercise duration between hour 0 and hour 4, when patients were given either placebo or L-arginine capsules. On the final study day of each treatment period, the 0-h test was carried out with the previous TD-GTN in place (i.e., 24 h after application) and before the morning capsule (i.e., 9 to 10 h after the previous evening’s dose). The data at 4 h represent TWT 4 h after TD-GTN reapplication and administration of the study capsule. In comparison with placebo capsules, L-arginine significantly prolonged TWT to moderate angina at 4 h and 24 h after TD-GTN application (Table 1, Fig. 1). This indicates the prevention of nitrate tolerance during L-arginine administration. There were no significant differences in the heart rate or systolic BP at rest or during exercise during the two phases of the investigation.

DISCUSSION

The organic nitrates remain important agents in the management of patients with angina pectoris. There is, however, a major drawback with nitrate therapy: it provides incomplete protection because it is not possible to provide antianginal and anti-ischemic effects throughout 24 h each day, owing to the rapid development of tolerance (2,3). Dosing strategies that provide a period of washout have documented that antianginal and anti-ischemic effects can be documented for 12 h with TD-GTN (4,19) and for 12 to 14 h with oral isosorbide-5-mononitrate given either as a controlled release preparation (20) or as eccentric dosing with immediate release isosorbide-5-mononitrate (21,22). The intermittent regimen with nitrates may result in rebound ischemia during the nitrate-free period. This has been shown in studies with TD-GTN (4,23,24) but has not been studied carefully with oral nitrate preparations.

The present study documents that L-arginine alone does not improve exercise duration in patients with stable angina. However, during continuous TD-GTN therapy, a regimen that has been shown to induce tolerance within 24 h, L-arginine improves exercise performance for up to 24 h. This is in contrast with the complete tolerance seen during continuous TD-GTN alone, when placebo capsules were taken. Of note, this is the first reported regimen in which significant antianginal effects were observed throughout the 24 h during continuous nitrate therapy. The results of this

Table 1. Treadmill Exercise Time to Moderate Angina (s)

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<tr>
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<th>Placebo</th>
<th>LA</th>
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<tr>
<td>Day 1</td>
<td></td>
<td></td>
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<tr>
<td>0 h</td>
<td>289 ± 83</td>
<td>287 ± 81</td>
</tr>
<tr>
<td>4 h</td>
<td>307 ± 106</td>
<td>303 ± 97</td>
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<tr>
<td>PL + TD-GTN</td>
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<td>Days 5–10</td>
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<tr>
<td>0 (24 h)</td>
<td>304 ± 110*</td>
<td>274 ± 93</td>
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<tr>
<td>4 h</td>
<td>314 ± 95†</td>
<td>287 ± 96</td>
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Data are mean ± SD. *p < 0.05 (confidence interval: 2.7, 51.5); †p < 0.05 (confidence interval: 1.3, 49.4).
LA = L-arginine; PL = placebo; TD-GTN = transdermal nitroglycerin patches.
Investigation support observations from two studies with L-arginine. One of these documented improved exercise tolerance in patients with angina treated with nitrates (25), and the other showed reversal of tolerance that developed during intravenous GTN therapy (26).

Mechanisms of tolerance. Recent advances have been made in our understanding of the etiology of nitrate tolerance. Important observations demonstrated that vessels exposed to nitrates, particularly GTN, developed specific biochemical abnormalities, including increased superoxide anion generation and endothelin-I content (13,27). Increased production of superoxide anion was found to be dependent on the presence of the endothelium, and animal studies revealed that tolerance to GTN was, in large part, an endothelium-dependent process (13,15). These observations have led to clinical investigations demonstrating that continuous, and intermittent, GTN therapy are both associated with the development of endothelial dysfunction in the coronary and peripheral circulation (28–30). Clinical investigations have also been carried out suggesting that the development of tolerance can be prevented by concurrent therapy with antioxidant vitamins (31,32).

Free radical generation and tolerance. This body of work has led to the free radical hypothesis of nitrate tolerance, a hypothesis that suggests that tolerance is associated with increased vascular superoxide anion production and decreased NO bioavailability. Important questions remain concerning the exact mechanism triggering increased superoxide production by the endothelium. The source of nitrate-induced superoxide anion production was originally reported to be membrane bound (NADH and NADPH) oxidases, a process that was found to be promoted by angiotensin II (15,33,34). More recently, endothelial NO synthase (NOS) has also been reported as a source of increased superoxide anion production in response to GTN treatment (16,17). Evidence derived from animal experiments suggests that GTN therapy causes an uncoupling of NOS, such that the enzyme preferentially utilizes molecular oxygen with the resultant production of superoxide anion. The mechanism of this triggering remains unclear and may be multifactorial. Importantly, it has been demonstrated that the NO donor GTN activates NOS and, thus, increases L-arginine requirements (14). This increase in L-arginine demand leads to relative L-arginine depletion, resulting in the uncoupling of NOS and increased superoxide anion generation (14,17). A number of lines of evidence suggest that changes in L-arginine transport mechanisms during therapy with GTN may be important. In vitro studies have demonstrated that NO donors reduce the transport of L-arginine, leading to depletion of this substrate (14,35). It is now recognized that the arginine transporter and NOS are co-localized within caveolae of the endothelial cell and that changes in arginine concentration may not be uniform throughout the endothelial cell (17,36). These observations have led to the arginine-depletion hypothesis of nitrate tolerance. In this case, therapy with nitrates leads to L-arginine depletion within caveoli of endothelial cells with resultant uncoupling of NOS and increased superoxide anion production. In vitro studies have demonstrated that L-arginine supplementation prevents the uncoupling of NOS during nitrate exposure and modifies the development of tolerance (35). Of note, there is some experimental evidence to suggest that L-arginine has antioxidant effects as a scavenger for superoxide anion (37). This characteristic might also play a role in modifying redox responses to GTN therapy.

It is important to point out that the majority of experimental data concerning increased free radical production during nitrate exposure have involved GTN. Whether increased free radial production occurs with other organic nitrates remains unclear. Reports in animal models and in humans have suggested that pentaerythritol tetryranitrate may not lead to increased free–radical formation (38). As yet, there are no clear data concerning the effect of isosorbide dinitrate and the isosorbide mononitrates on the generation of oxygen–free radicals.

If NO donated by the organic nitrates oxidatively injures the arginine transport system, why does endogenous NO not lead to such abnormalities? It has been demonstrated that NOS agonists, such as bradykinin and acetylcholine, increase L-arginine transport instead of leading to the inhibition seen with GTN. One explanation for the lack of inhibition of the transport system with NOS agonists could be that the amount of NO produced upon NOS activation is far less than the amount released by NO donors. Another possible explanation for the lack of inhibition of L-arginine transport with these agonists is that L-arginine is converted to the intermediate 4-N(G)-hydroxyl-L-arginine before formation of NO. L-hydroxyl arginine is an antioxidant and an inhibitor of arginase (39,40), and this may provide protection from oxidation by newly formed NO.

This is the first study demonstrating an intervention that would provide beneficial effects of an organic nitrate on exercise performance for up to 24 h. It is interesting to note that an intervention designed to prevent abnormalities in NOS function during GTN treatment may also be able to prevent the development of tolerance. This has been suggested by a recent observation by one of us who was examining the impact of supplemental folate on both endothelial function and vascular tolerance (41,42). The observation that supplemental L-arginine is associated with continued clinical effectiveness of TD-GTN supports the L-arginine-depletion hypothesis as a significant factor in the development of nitrate tolerance. The small number of patients in this pilot study is a significant weakness. The improvement in exercise performance 4 h and 24 h after TD-GTN during continuous therapy, when patients were receiving L-arginine, is of statistical significance, but the confidence intervals are wide. The data suggest that L-arginine supplementation modified the development of tolerance to GTN, but it cannot be stated that this modification completely prevents tolerance. In order to make the
latter statement, it would be necessary to compare the initial responses to TD-GTN and placebo in a blinded fashion in these patients. Unfortunately, placebo TD-GTN was not available to us.

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REFERENCES