Oral L-Arginine in Patients With Coronary Artery Disease on Medical Management

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Background—Vascular nitric oxide (NO) bioavailability is reduced in patients with coronary artery disease (CAD). We investigated whether oral L-arginine, the substrate for NO synthesis, improves homeostatic functions of the vascular endothelium in patients maintained on appropriate medical therapy and thus might be useful as adjunctive therapy.

Methods and Results—Thirty CAD patients (29 men; age, 67±8 years) on appropriate medical management were randomly assigned to L-arginine (9 g) or placebo daily for 1 month, with crossover to the alternate therapy after 1 month off therapy, in a double-blind study. Nitrogen oxides in serum (as an index of endothelial NO release), flow-mediated brachial artery dilation (as an index of vascular NO bioactivity), and serum cell adhesion molecules (as an index of NO-regulated markers of inflammation) were measured at the end of each treatment period. L-Arginine significantly increased arginine levels in plasma (130±53 versus 70±17 μmol/L, P<0.001) compared with placebo. However, there was no effect of L-arginine on nitrogen oxides (19.3±7.9 versus 18.6±6.7 μmol/L, P=0.546), on flow-mediated dilation of the brachial artery (11.9±6.3% versus 11.4±7.9%, P=0.742), or on the cell adhesion molecules E-selectin (47.8±15.2 versus 47.2±14.4 ng/mL, P=0.601), intercellular adhesion molecule-1 (250±57 versus 249±57 ng/mL, P=0.862), and vascular cell adhesion molecule-1 (567±124 versus 574±135 ng/mL, P=0.473).

Conclusions—Oral L-arginine therapy does not improve NO bioavailability in CAD patients on appropriate medical management and thus may not benefit this group of patients. (Circulation. 2000;101:2160-2164.)

Key Words: atherosclerosis ■ coronary disease ■ endothelium ■ nitric oxide

The coronary response to the endothelium-dependent vasodilator acetylcholine is depressed in patients with coronary artery disease, as it is in patients with early atherosclerosis or even with risk factors for atherosclerosis despite normal-appearing coronary angiograms.1–5 Basal and acetylcholine-stimulated release of nitric oxide (NO) from the endothelium is reduced in these patients,5 which could be due either to depressed synthesis of NO or to excess degradation of NO by reactive oxygen species to biologically inert or even toxic molecules. L-Arginine is a semiessential amino acid that serves as the substrate for the enzyme NO synthase, which converts arginine to citrulline and NO.6 Normally, L-arginine is not rate limiting in this reaction; the Kₘ for NO synthase is in the micromolar range, whereas intracellular levels of arginine are in the millimolar range. Under certain conditions, however, L-arginine administration can improve endothelium-dependent dilator responsiveness. Thus, after intracoronary infusion of L-arginine in patients with coronary artery disease, constrictor responses of epicardial arteries to acetylcholine were converted to dilator responses and flow responses to this agonist were improved.7–11

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Practical application of L-arginine therapy over time requires oral administration, which has been shown to improve brachial artery flow-mediated dilation, an index of NO bioactivity,12 in hypercholesterolemic subjects13 and coronary blood flow responses to acetylcholine in patients without significant coronary disease.14 Furthermore, benefit of L-arginine therapy might be additive to that of appropriate medical management previously determined to improve morbidity and mortality risk of patients with coronary artery disease.15 In this regard, oral administration of L-arginine 6 g/d for 3 days improved exercise duration of 12 coronary artery disease patients on medical management, but the mechanism of therapeutic benefit was not determined.16 We therefore reasoned that oral administration of L-arginine could improve endothelium-dependent dilator responsiveness and other homeostatic properties of the vasculature potentially regulated by NO in patients with coronary artery disease who are otherwise appropriately managed with medical therapy.
Methods

Patient Selection
Thirty-five patients (33 men; average age 67±8 years, range 48 to 78 years) with angiographically documented coronary artery disease (>70% stenosis in ≥1 coronary artery at the time of diagnostic catheterization) were enrolled in this study. All patients were in Canadian Cardiovascular Society class I or II and continued taking medications throughout the study: aspirin (n=33), HMG-CoA reductase inhibitors (statins) (n=18), β-blockers (n=18), calcium channel blockers (n=10), ACE or angiotensin type 1 (AT1) receptor inhibitors (n=8), nonstatin lipid-lowering agents (n=5), and chronic nitrates (n=3). All patients took sublingual nitrates as needed for angina pectoris. Ten patients were taking oral hypoglycemic agents for diabetes mellitus. Ten patients had ischemic ST-segment depression during treadmill exercise. No patient had symptoms of heart failure or evidence of depressed left ventricular function (<40% ejection fraction determined by radionuclide angiography or echocardiography), recent (within 6 months) myocardial infarction, cardiomyopathy, valvular heart disease, or hypertension (blood pressure >160/100 mm Hg even after taking blood pressure medications). Two patients decided not to participate in the study after enrollment, 2 patients dropped out of the study during the L-arginine treatment phase because of nausea and stomach cramps, and 1 patient died suddenly after completion of the placebo phase (first phase of study). Thus, 30 patients completed the study. Their lipid profile on medications was as follows: total cholesterol 188±31 mg/dL, LDL cholesterol 115±31 mg/dL, HDL cholesterol 42±9 mg/dL, and triglycerides 146±96 mg/dL. The study was approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute, and all participants gave written informed consent.

Study Design
Patients were randomized to L-arginine 9 g (3 g 3 times a day with meals) or identical placebo capsules 3 times a day with meals, each for 1 month with 1 month off therapy before crossover to the other treatment. All patients were placed on a nitrate-restricted diet for 72 hours before each study to reduce the contribution of dietary nitrates to serum nitric oxide levels. After an overnight fast (except for water), patients returned to the clinical center for blood drawing and vascular studies at the end of each treatment period.

Laboratory Assays
Blood samples were coded so that investigators performing assays were blinded to patient identity and study sequence. Plasma and serum were separated by centrifugation and stored at −80°C until analysis. Arginine was measured in plasma by ion-exchange chromatography (Mayo Medical Laboratories). Growth hormone was determined in serum by a chemiluminescent enzyme immunoassay (Immulite, DPC). Insulin was measured in serum with a microparticle enzyme immunoassay (IMx, Abbott Laboratories). Nitrogen oxide levels were measured in serum by a chemiluminescent technique (Sievers Instruments, Inc). C-reactive protein (2-site chemiluminescent enzyme immunoassay assay; sensitivity 0.01 mg/dL; Immulite, DPC), interleukin-6 (sandwich enzyme immunoassay, R&D Systems, Inc), tissue factor (enzyme-linked immunomassay, American Diagnostica, Inc), and plasminogen activator inhibitor-1 (sandwich enzyme-linked immunoassay, Biopool) were measured as markers of vascular inflammation. The cell adhesion molecules E-selectin, P-selectin, ICAM-1, and VCAM-1 were measured by enzyme-linked immun massaays (R&D Systems, Inc) as markers of inflammation that are modulated in tissue culture experiments by NO.18

To further detect an effect of L-arginine on inflammation, blood samples for flow cytometry were processed to prepare leukocytes for immunofluorescence measurements with the Coulter Q-Prep workstation (Beckman Coulter Corp). Expression of the following integrin adhesion molecules was measured on monocytes, lymphocytes, and granulocytes: LFA-1 (DC11a), Mac-1 (CD11b), and VLA-4 (CD49d), as well as the selectin adhesion molecule E-selectin (CD62L).19 All monoclonal antibodies to cell-surface adhesion molecules were directly conjugated to FITC and were obtained from Immunotech (Beckman Coulter Corp). Flow cytometry was performed with the EPICS XL-MCL flow cytometer (Beckman Coulter Corp) equipped with a 15-mW, 488-nm argon ion laser. A total of ≥50 000 events per sample were analyzed. The lymphocytes, monocytes, and granulocytes were electronically isolated by a collection of a dual-parameter histogram of size and granularity (forward-angle light scatter versus log 90° light scatter). Fluorescence intensity of the measured adhesion molecule was expressed as mean channel units. Relative FITC log fluorescence was calculated with the histogram data. Flow cytometry settings remained constant for all data generated, and standard beads were used to calibrate the instrument.

Vascular Studies
Imaging studies of the left brachial artery were performed with a high-resolution ultrasound Hewlett-Packard 7.5-MHz linear-array transducer after 30 minutes of rest, based on the technique reported by Celermajer et al.20 After the clearest view of the brachial artery was found, the skin was marked and the arm kept in the same position throughout the study. Baseline measurements included brachial artery diameter and flow velocity measured by pulsed Doppler, with the range gate (1.5 mm) in the center of the artery. The system permitted a direct assessment of the angle between the bloodstream and the intersecting ultrasound beam, which was then used to calculate blood flow velocity. Endothelium-dependent vasodilation was assessed by measurement of the change in diameter of the brachial artery during reactive hyperemia created by an inflated cuff (250 mm Hg for 5 minutes) on the forearm. After cuff deflation, flow velocity was measured for the first 15 seconds, and arterial diameter was recorded continually for the next 60 seconds. Fifteen minutes later, repeat baseline diameter and flow velocity measurements were made, followed by nitroglycerin spray (0.4 mg) under the tongue to assess endothelium-independent vasodilation. Three minutes later, arterial diameter and flow velocity measurements were recorded. Arterial diameter was measured in millimeters from the artery-blood interface on both the anterior and posterior walls, coincident with the R waves on the ECG, at 2 sites along the artery for the 3 cardiac cycles, with these 6 measurements averaged. During hyperemia, 6 measurements of arterial diameter were averaged during maximum dilation between 50 and 70 seconds after cuff deflation. We calculated blood flow by multiplying the velocity-time integral of the Doppler flow signal by the heart rate and the cross-sectional area of the vessel.

Reproducibility of Study Parameters
In a recently published study21 of 28 healthy postmenopausal women who underwent studies while not taking any hormone or other therapies on 3 occasions, each separated by 12 weeks, we computed coefficients of variation for study parameters as the square root of the pooled within-subject variance divided by the mean of the averages over all subjects for each parameter: flow-mediated brachial artery dilation 0.623; nitrogen oxides 0.365; E-selectin 0.226; ICAM-1 0.159; and VCAM-1 0.178.

Statistical Analysis
Measurements are expressed as mean±SD. The 2-sided paired Student’s t test was used to compare changes in vascular responses and laboratory values between L-arginine and placebo treatments, with P<0.05 an indicator of statistical significance. The primary study comparison predefined in advance of data collection was flow-mediated dilation of the brachial artery with L-arginine treatment compared with placebo. All other comparisons were regarded as secondary, and no adjustments to probability value for multiple comparisons were performed.
TABLE 1. Effects of L-Arginine on Brachial Artery Endothelial Function

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>L-Arginine</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachial artery diameter, mm</td>
<td>4.60±0.71</td>
<td>4.38±0.61</td>
<td>0.22</td>
</tr>
<tr>
<td>Basal</td>
<td>4.12±0.70</td>
<td>3.96±0.64</td>
<td>0.12</td>
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<tr>
<td>Hyperemia</td>
<td>730±247</td>
<td>714±270</td>
<td>0.52</td>
</tr>
<tr>
<td>Brachial artery flow, mL/min</td>
<td>125±57</td>
<td>115±52</td>
<td>0.34</td>
</tr>
<tr>
<td>Basal</td>
<td>125±57</td>
<td>115±52</td>
<td>0.34</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>730±247</td>
<td>714±270</td>
<td>0.52</td>
</tr>
<tr>
<td>Flow-mediated dilation, %</td>
<td>11.9±6.3</td>
<td>11.4±7.9</td>
<td>0.74</td>
</tr>
<tr>
<td>Nitroglycerin, %</td>
<td>19.8±14.4</td>
<td>20.0±9.7</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Data are mean±SD.

Results

Effects of L-Arginine on Vasomotor Function

L-arginine therapy nearly doubled arginine levels in plasma (130±53 versus 70±17 μmol/L, P<0.001) compared with placebo. Evidence of a pharmacological response to L-arginine was shown by trends toward increases in serum levels of growth hormone (0.68±1.30 versus 0.44±0.79 ng/mL, P=0.193) and insulin (14.1±8.5 versus 11.5±6.6 μU/mL, P=0.140). However, there were no significant differences in serum nitrogen oxides (19.3±7.9 versus 18.6±6.7 μmol/L, P=0.546), and there was no effect of L-arginine treatment on brachial artery diameters, flow-mediated dilation, or nitroglycerin-induced dilation (Table 1). In our study of 30 patients, we could detect as statistically significant a true treatment difference in flow-mediated dilation of ≥4.2% between L-arginine and placebo treatment with a 2-sided paired t test at α=0.05 and 80% power.

Effects of L-Arginine on Markers of Inflammation

There was no effect of L-arginine on serum levels of the cell adhesion molecules E-selectin (47.8±15.2 versus 47.2±14.4 ng/mL, P=0.601), P-selectin (98.2±30.5 versus 94.6±28.8 ng/mL, P=0.193), intercellular adhesion molecule-1 (ICAM-1; 250±57 versus 249±57 ng/mL, P=0.86), and vascular cell adhesion molecule-1 (VCAM-1; 567±124 versus 574±135 ng/mL, P=0.26) compared with placebo. Other markers of inflammation were likewise unaltered by L-arginine therapy: interleukin-6 (4.41 versus 4.76 pg/mL, P=0.588), C-reactive protein (0.40±0.34 versus 0.36±0.27 mg/dL, P=0.579), tissue factor antigen (232.6±135.6 versus 252.9±175.9 pg/mL, P=0.228), and plasminogen activator inhibitor-1 antigen (27.2±16.6 versus 30.7±18.6 ng/mL, P=0.194). There was no effect of L-arginine on the expression of cell adhesion molecules on circulating inflammatory cells (Table 2).

Discussion

In our study, oral L-arginine treatment (9 g/d for 1 month) of patients with chronic stable coronary artery disease significantly increased plasma arginine levels. However, there was no significant effect of L-arginine on brachial artery flow-mediated dilation, an in vivo bioassay for endothelial NO release.12 Because of the variance of our measurements, possibly due to the confounding effects of medical therapy, we could have missed a small improvement in this response with L-arginine therapy. However, there was no effect of L-arginine on 2 other measures of NO bioactivity, serum nitrogen oxides and cell adhesion molecules reduced by NO in cell culture experiments.18 Furthermore, there was no effect of L-arginine on other soluble and cell-based markers of inflammation. Stimulatory effects of L-arginine on insulin, although marginal in our study population, could be of atherogenic concern in larger populations of coronary artery disease patients.

It is possible that an effect of oral L-arginine on these markers of NO bioactivity might have been detected in study participants had we stopped aspirin and vasoactive and lipid-lowering medications during the study. However, we chose this study design because we felt that discontinuation of appropriate medical therapy for 3 months was medically unwise in these patients (10 patients had inducible ischemia by treadmill exercise testing) and that demonstration of a positive effect of L-arginine on NO bioactivity and vascular homeostasis in patients on medical therapy previously shown to reduce cardiovascular risk would be of greater clinical relevance in the management of patients with coronary artery disease.

It is possible that had a more hypercholesterolemic population of patients with coronary disease been recruited for our study, an effect of L-arginine on NO bioactivity would have been detected. In this regard, chronic administration of L-arginine to hypercholesterolemic rabbits and humans improved endothelium-dependent vasorelaxation.13,22 Oral administration of L-arginine to hypercholesterolemic rabbits reduced vascular release of superoxide and ions and restored NO production and endothelial function,23 in addition to reducing monocyte adherence to vascular surfaces,24 consistent with an anti-inflammatory effect of this therapy. However, current guidelines of the National Cholesterol Education Program dictate aggressive cholesterol reduction in patients with coronary artery
disease, which is supported by reductions in morbidity and mortality reported in 3 secondary prevention trials using statin therapy.25–27 In the present study, most patients were taking statin lipid-lowering therapy, with an average LDL cholesterol level of 115 mg/dL for the group. Thus, the robust flow-mediated dilation seen in our patients may reflect relatively normalized endothelial-dependent vasodilator responsiveness previously demonstrated with statin lipid-lowering therapy.28–31

In addition to the confounding effects of medical therapy, a potential explanation for failure to demonstrate vascular effects of L-arginine in the present study is that the dose used (9 g/d) was insufficient. However, higher doses have been associated with side effects of nausea, stomach cramps, and diarrhea.13 Indeed, in the present study, 2 patients dropped out of the study during the L-arginine treatment phase because of nausea and stomach cramps. Furthermore, in a study of hypercholesterolemic subjects,13 administration of L-arginine 21 g/d for 1 month caused the same relative increase in serum L-arginine levels as was seen in our study group treated with 9 g/d for the same duration of administration. Another potential limitation of our study is that levels of asymmetric dimethylarginine (ADMA) were not measured in the plasma of our participants. Elevated levels of this competitive inhibitor of NO synthase have been reported in patients with risk factors for atherosclerosis.32 Thus, it is possible that had levels of ADMA been found to be low in our patients, improvement in endothelial function with L-arginine would not have been expected. Nevertheless, given the current interest in L-arginine as a therapeutic supplement to prevent atherosclerosis, the present study suggests that L-arginine may not benefit the general population of patients with coronary artery disease on appropriate medical management.

In conclusion, we did not find evidence that L-arginine, when administered for 1 month to patients with coronary artery disease on appropriate medical management, augments endothelial NO bioavailability or reduces markers of inflammation associated with coronary artery disease. Thus, L-arginine may not be of general benefit to patients with chronic stable coronary artery disease on appropriate medical management in the protection from progression or clinical expression of atherosclerosis.

Acknowledgments

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References


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