Effects of inhibition of nitric oxide synthesis in patients with coronary artery disease and stable angina

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Aims
Inhibition of nitric oxide synthesis causes a decrease in the basal diameter of normal distal epicardial coronary arteries in normal subjects. The effects of inhibition of nitric oxide in atheromatous coronary arteries is unknown. This study assessed the effects of the inhibition of nitric oxide synthesis in epicardial coronary arteries in patients with coronary artery disease.

Methods and results
The effects of an intracoronary infusion of NG-monomethyl-L-arginine (LNMMA, an inhibitor of nitric oxide synthesis), were studied in 13 patients with chronic stable angina and coronary artery disease. The diameter of angiographically normal proximal and distal segments and coronary stenoses was measured by quantitative angiography. In response to an LNMMA infusion of 16 μmol min⁻¹ for 4 min there was a significant reduction (P<0.01) in the luminal diameter of the distal segments of diseased arteries (from 1.32 ± 0.07 to 1.17 ± 0.06 mm) and at the site of stenosis (from 1.15 ± 0.22 to 1.06 ± 0.20 mm), but no change (P=NS) in the luminal diameter of the proximal segments (from 3.16 ± 0.12 to 3.08 ± 0.14 mm) of diseased arteries.

Conclusions
The average effect of inhibition of basal nitric oxide synthesis in epicardial coronary arteries in patients with stable angina and coronary artery disease was only distal constriction. Coronary stenoses constricted at the highest LNMMA concentration.

Key Words: Endothelium, nitric oxide, coronary artery disease, endothelium-derived relaxing factor.
if they had diabetes mellitus, recent myocardial infarction (<6 months), left ventricular hypertrophy (on echocardiography), left ventricular dysfunction (left ventricular ejection fraction <50%) or valvular heart disease. Antianginal medication was stopped 24 h before the study. The patients were allowed to use sublingual nitroglycerin as necessary, but no study was performed within 3 h of its administration. The protocol was approved by the Research Ethics Committee and each patient gave written informed consent.

Protocol

Following the diagnostic coronary angiogram, an optimal radiographic projection was selected and kept constant for subsequent angiograms. The artery studied was chosen to comply with the Research Ethics Committee’s requirements that coronary stenoses causing approximately >75% luminal diameter reduction be avoided. Seventeen stenoses with luminal diameter reduction 20-2% to 70-0% were examined.

Protocol 1

Two ECG leads were monitored continuously throughout the study. During a preliminary study in two patients we infused very low doses of LNMMA (Cilnafia) into the coronary arteries (1 µmol. min⁻¹, 2 µmol. min⁻¹, 4 µmol. min⁻¹) for 4 min. There were no adverse effects, but in one of these patients constriction of the distal coronary artery and increased blood pressure was observed at the 4 µmol. min⁻¹ dose. Nine patients received a single 2 min infusion of 0-9% saline (2 ml. min⁻¹) followed by a 4 min infusion of LNMMA (4 µmol in 2 ml. min⁻¹) in saline, using a syringe pump, followed by an intracoronary bolus dose of nitroglycerin (250 µg in 2 ml of saline).

Protocol 2

Two ECG leads were monitored continuously throughout the study. Four patients received a single 2 min infusion of 0-9% saline (2 ml. min⁻¹) followed by a 4 min infusion of incremental doses of LNMMA (2, 4, 8, 16 µmol. min⁻¹) in saline, using a syringe pump, followed by an intracoronary bolus dose of nitroglycerin (250 µg in 2 ml of saline).

Femoral arterial pressure and heart rate were recorded during the last 30 s of each infusion period. Angiography was performed with a hand injection of 6-8 ml non-ionic contrast medium at baseline, immediately after each infusion and 2-3 min after nitroglycerin. Before each angiogram, the catheter was emptied to avoid bolus administration of the infusate.

Quantitative coronary angiography

The arterial segments in each frame were analysed in random order using quantitative computerized analysis with an automated edge contour detection analysis system (Computerized Angiographic Analysis System [CAAS], Version 2V2; Pie Data Medical)¹⁹⁹. End-diastolic frames from each arteriogram were selected for analysis. The angiographic catheter was used as a scaling device and this, together with the pincushion-distortion correction, allowed the diameters to be recorded as absolute values (expressed in mm). Recorded variables at baseline and after saline, LNMMA, and nitrate administration were: (1) the luminal diameter of angiographically normal proximal and distal segments; the proximal left anterior descending coronary artery diameter was measured just beyond the origin of the artery, and the distal diameter was measured just distal to the second diagonal branch; the proximal left circumflex coronary artery diameter was measured just beyond the origin of the artery, and the distal diameter just beyond the origin of the second obtuse marginal branch; the proximal right coronary artery diameter was measured just beyond the origin of the artery, and the distal diameter just beyond the posterior descending branch; (2) the minimum luminal diameter at the site of coronary stenosis.

Quantitative analysis of coronary arteriograms was carried out by two independent observers, who blindly reanalysed the films at a remote time for reproducibility of the method. No significant intra- or interobserver variability was found (analysis of variance F=0.35, P=0.80).

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). When serial changes in the heart rate, blood pressure and arterial diameter were compared within the group and between the groups, analysis of variance for repeated measures was used. Student's t-test was used to compare paired or unpaired data. Discrete data were analysed by the χ² test. P values of <0.05 (two tailed) were considered to indicate statistical significance.

Results

Six patients had hypercholesterolaemia (serum cholesterol level >220 mg. dl⁻¹), seven were hypertensives and six were smokers.

**LNMMA infusion 4 µmol. min⁻¹**

(*protocol 1*)

The heart rate remained unchanged during LNMMA infusion (72 ± 1.3 and 74 ± 1.7 beats. min⁻¹ at baseline and after LNMMA respectively), but there was a significant change in systolic blood pressure (134 ± 6.3 and 140 ± 6.0 mmHg at baseline and after LNMMA respectively, P<0.01), and one patient developed chest pain.

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The response to LNMMA varied markedly between individual patients (Fig. 1). There was no significant change in the luminal diameter of proximal segments or at the site of stenosis, but there was a significant reduction in the luminal diameter of distal segments (Table 1). A greater proportion of distal segments than of proximal segments showed a reduction in luminal diameter of more than 10% in response to LNMMA and an increase of greater than 10% in response to nitrates (Table 2).

**LNMMA infusion 2 to 16 μmol. min⁻¹ (protocol 2)**

During the highest LNMMA concentration, the heart rate remained unchanged (66 ± 2.8 at baseline and 69 ± 2.5 beats. min⁻¹ after LNMMA), but there was a significant change in systolic blood pressure (145 ± 15 at baseline and 156 ± 13 mmHg after LNMMA, P<0.05), and one patient developed chest pain.

There was no significant change in luminal diameter in the proximal segments (Table 3). However, there was a significant reduction in the luminal diameter of distal segments at LNMMA concentrations of 4, 8 and 16 μmol. min⁻¹ (Table 3, Fig. 2), and a greater proportion of distal segments than of proximal segments showed a reduction in luminal diameter of more than 10% (Table 2). At 16 μmol. min⁻¹, the magnitude of vasoconstriction was greater in distal than in proximal segments (P<0.05), and the coronary stenoses showed significant constriction (P<0.01) (Table 3, Fig. 2).

**Discussion**

In this study we examined the effects of LNMMA in patients with coronary artery disease and stable angina. The LNMMA-induced constriction and nitrate-induced dilatation were greater in distal than in proximal segments and there was greater variation in response between individuals, particularly at 4 μmol. min⁻¹. The proximal segments showed no significant constriction at any LNMMA concentration, but dilated in response to nitrate. The stenoses showed significant constriction only at the highest infused concentration of LNMMA (16 μmol. min⁻¹).

Differential reactivity between proximal and distal segments, particularly when expressed as percentage change, has been found in response to many vasoactive stimuli and appears to be an inherent characteristic of human coronary arteries which is not disease dependent. This differential activity could reflect a difference in the ratio of the endothelial cells to smooth muscle cells. Constriction in the distal vessels presumably indicates that there is basal production of nitric oxide and therefore that the endothelium in those vessels is synthesizing nitric oxide in spite of proximal disease. The lack of response to inhibition of nitric oxide synthesis in the

**Figure 1** Graphs showing luminal diameter changes in proximal segments (top panel), distal segments (middle panel), and coronary stenoses (bottom panel), in patients with coronary artery disease. Responses in individual patients to intracoronary infusion of 4 μmol. min⁻¹ LNMMA and intracoronary infusion of nitroglycerin (nitrate) are expressed as a percentage change from baseline.
Table 1 Reactivity of proximal and distal segments and coronary stenoses to intracoronary LNMMA (4 \mu mol . min^{-1}) and nitrates (protocol 1)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Minimum luminal diameter (mm)</th>
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<th>Minimum luminal diameter (mm)</th>
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<th>Minimum luminal diameter (mm)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>LNMMA</td>
<td></td>
<td>Nitrates</td>
<td></td>
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<tr>
<td>Proximal (n=15)</td>
<td>2.95 ± 0.16</td>
<td>2.94 ± 0.16 (-0.5 ± 0.6%)</td>
<td>2.89 ± 0.16 (-2.0 ± 1.6%)</td>
<td>3.27 ± 0.15 (11.9 ± 2.4%)†</td>
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<tr>
<td>Distal (n=23)</td>
<td>1.44 ± 0.06</td>
<td>1.45 ± 0.06 (0.8 ± 0.7%)</td>
<td>1.33 ± 0.07 (-7.4 ± 2.5%)*</td>
<td>1.75 ± 0.07 (22.7 ± 3.0%)†</td>
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<td>Stenoses (n=11)</td>
<td>1.64 ± 0.12</td>
<td>1.65 ± 0.12 (0.5 ± 0.8%)</td>
<td>1.60 ± 0.12 (-2.3 ± 1.5%)</td>
<td>1.90 ± 0.14 (17.1 ± 3.5%)†</td>
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*P<0.05 compared with the saline infusion.
†P<0.05 compared with the saline infusion.
Percentage change from baseline in parentheses.

Table 2 Proportion of proximal and distal segments and coronary stenoses with dynamic reactivity (≥10% change from baseline) in response to intracoronary LNMMA and nitrates

<table>
<thead>
<tr>
<th></th>
<th>Proximal segments</th>
<th>Distal segments</th>
<th>Stenoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNMMA (4 \mu mol . min^{-1})</td>
<td>2/15</td>
<td>11/23*</td>
<td>1/11**</td>
</tr>
<tr>
<td>LNMMA (16 \mu mol . min^{-1})</td>
<td>1/8</td>
<td>5/11†</td>
<td>2/6</td>
</tr>
<tr>
<td>Nitrates</td>
<td>6/15</td>
<td>17/23*</td>
<td>8/11</td>
</tr>
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</table>

*P<0.01 compared with proximal segments.
**P<0.01 compared with distal segments.
†P<0.05 compared with proximal segments.

Proximal segments of patients with coronary artery disease may have been a reflection of proximal endothelial damage related to subtle non-angiographically detectable lesions. The fact that proximal segments and stenoses responded to nitroglycerin, even when there was little or no response to inhibition of nitric oxide synthesis, suggests that these segments maintained the physical ability to contract. It is therefore likely that the lack of response to LNMMA is due to the loss of the site or mechanism of nitric oxide synthesis. Although at lower concentrations the response of stenoses was similar to that of proximal segments (in which they were mostly located), at 16 \mu mol . min^{-1} constriction occurred. This raises the possibility that basal nitric oxide production is preserved at the site of stenosis. Preserved local nitric oxide production is likely to originate from the endothelial cells of the vasa vasorum which is more abundant around atherosclerotic plaque.

In a recent study, Lefroy et al. examined the effects of LNMMA in the human circulation and on the coronary vascular responses to acetylcholine and sodium nitroprusside in patients with normal coronary arteries. They showed that the inhibition of nitric oxide synthesis in human coronary circulation caused a decrease in basal distal coronary artery diameter and basal coronary blood flow, indicating that there is a small basal release of nitric oxide in the distal epicardial coronary arteries and resistive vessels. Furthermore, they showed that distal epicardial coronary artery dilation in response to acetylcholine is nitric-oxide dependent, but coronary resistive vessel dilation is not. Chu et al., in an experimental model, showed that after systemic doses of LNMMA sufficient to raise the blood pressure and decrease the heart rate, there was a reduction in proximal epicardial coronary artery diameter of between 5.5% and 8%. In previous studies, indirect evidence for the role of nitric oxide production in the human coronary circulation has been obtained with agents such as acetylcholine, substance P, and serotonin, which are known to stimulate endothelial nitric oxide production.

These studies have shown that intracoronary infusion of each of these agents causes dilation of normal coronary arteries and that the effect at a given dose is more marked in the distal than in the proximal segments. These findings, together with our data,
suggest a difference in behaviour between the proximal and distal segments, which may be related to their potential for nitric oxide generation. Differences in this potential might also explain the differences in response between individual patients. Those patients with a large response to LNMMA infusion and only a small incremental response to nitrate may have had a high basal level of nitric oxide generation (and/or more complete inhibition of nitric oxide synthesis by LNMMA), in contrast to those patients with a small response to LNMMA infusion and a large response to nitrate.

The results of our study indicate a dose-dependent effect of basal nitric oxide production on the tone of epicardial coronary arteries in humans in the resting state. The concentration of LNMMA used in our present study was sufficient to increase systemic arterial pressure and therefore certainly had an inhibitory effect on nitric oxide synthase. Although there is a basal production of nitric oxide by diseased coronary arteries, under conditions which stimulate endothelial nitric oxide production, the response to stimulation could be sufficiently impaired to prevent an adequate fall in coronary vascular resistance, with concomitant limitation of coronary flow increase and consequent myocardial ischaemia[26,27].

Since an inhibitor of nitric oxide synthesis had not previously been infused in patients with coronary artery disease, we elected initially to infuse very low doses of LNMMA into the coronary arteries of the first two patients. We did not infuse doses higher than 16 μmol . min⁻¹, because the increase in blood pressure observed in these patients indicated that a sufficient dose of LNMMA had been delivered to the coronary circulation. In our study we found that coronary stenoses constricted only at the highest LNMMA concentration. This is consistent with low basal production of nitric oxide by endothelium which has been damaged by the atherosclerotic process, and the lower inherent reactivity of proximal segments in which the stenoses were located.

In conclusion, in patients with chronic stable angina, coronary artery disease changes the response of proximal segments to inhibition of nitric synthesis, but not the response of distal segments. The change in response is compatible with disease induced changes in endothelium and endothelial function.

References
Nitric oxide in coronary artery disease


