Long-term Inhibition of NO Synthesis Promotes Atherosclerosis in the Hypercholesterolemic Rabbit Thoracic Aorta

PGH₂ Does Not Contribute to Impaired Endothelium-Dependent Relaxation

Kenshin Naruse, Kiyokazu Shimizu, Masahito Muramatsu, Yukio Toki, Yutaka Miyazaki, Kenji Okumura, Hidekazu Hashimoto, Takayuki Ito

Abstract We examined whether prostaglandin (PG) H₂, as an endothelium-dependent contracting factor, or the disturbed production of endothelium-derived relaxing factor, impairs endothelium-dependent relaxation and whether long-term inhibition of nitric oxide (NO) synthesis aggravates atherosclerosis in hypercholesterolemic rabbits. Male New Zealand White rabbits were fed one of the following diets: (1) standard chow; (2) 2% cholesterol-supplemented chow; (3) standard chow with 80 μg/mL N⁴-nitro-L-arginine methylester (L-NAME), an NO synthetase inhibitor, in their drinking water; or (4) 2% cholesterol-supplemented chow with 80 or 160 μg/mL L-NAME in their drinking water. The rabbits were fed these diets for 8 or 12 weeks. Then aortic rings were obtained and changes in isometric tension were recorded. Intimal atherosclerotic areas of the thoracic aortas were subsequently measured by planimetry. The cholesterol-supplemented diet significantly impaired endothelium-dependent aortic relaxation to acetylcholine. Pretreatment with the thromboxane A₂/PGH₂ receptor antagonist ONO-3708 did not reverse this impaired response. Vessels from both normocholesterolemic and hypercholesterolemic rabbits given L-NAME showed more impaired endothelium-dependent relaxation than those from their dietary counterparts not given L-NAME. Morphometric analysis revealed marked enlargement of intimal atherosclerotic areas in aortas from L-NAME–treated hypercholesterolemic rabbits compared with those from untreated hypercholesterolemic rabbits. These findings suggest that PGH₂ does not contribute to impaired endothelium-dependent relaxation and that long-term administration of L-NAME promotes atherosclerosis by inhibition of NO synthesis in the hypercholesterolemic rabbit thoracic aorta. (Arterioscler Thromb. 1994;14:746-752.)

Key Words • endothelium-derived relaxing factor • atherosclerosis • nitric oxide synthetase inhibitor

The endothelium produces various substances such as endothelium-derived contracting factors (EDCs)¹⁻² and endothelium-derived relaxing factor (EDRF)²⁻⁴ and controls vascular smooth muscle tone. Several substances, such as thromboxane (TX) A₂,⁵⁻⁶ superoxide anion,⁷ endothelin,⁸ and prostaglandin (PG) H₂,⁹⁻¹² may be candidates for EDCFs and are considered responsible for various pathological disorders. We have reported that increased release of PGH₂ as an EDCF, not decreased EDRF activity, is responsible for impaired endothelium-dependent relaxation in response to acetylcholine (Ach) in thoracic aortas of old Wistar-Kyoto rats (WKYs),⁹,¹⁰ spontaneously hypertensive rats (SHRs),⁹,¹¹ and diabetic rats.¹² These observations suggest that PGH₂ may also play an important role in the impaired endothelium-dependent relaxation induced by atherosclerosis.

The main component of EDRF has been identified as either nitric oxide (NO)¹³⁻¹⁵ or S-nitrosocysteine¹⁶ and is derived from the metabolism of L-arginine.¹⁵ EDRF not only is a potent vasodilator but also may have various effects on key events in atherosclerosis, such as platelet adherence, platelet aggregation,¹⁷⁻¹⁹ and vascular smooth muscle cell proliferation.²⁰ It may be possible to control atherosclerosis progression by modulating EDRF production.

One purpose of this study was to investigate whether PGH₂, as an EDCF, or the disturbed production of EDRF, could contribute to impaired Ach-induced relaxation in thoracic aortas in diet-induced atherosclerotic rabbits. The other purpose was to study the effect of long-term administration of N⁴-nitro-L-arginine methylester (L-NAME), an inhibitor of NO synthesis, on atherosclerosis progression in rabbit thoracic aortas.

Methods

Animals

Sixty-two male New Zealand White rabbits (2.4 kg to 3.0 kg) were used for the study. The animals were housed individually, and purified water (with added chloride for sterilization) was provided ad libitum as drinking water. Rabbits were fed either standard rabbit chow (standard diet group, n=21) or chow supplemented with 2% cholesterol (cholesterol diet group, n=41). The animals in the standard diet group were divided into two sets: those drinking untreated water (standard diet/normal water group, n=13) and those drinking water containing 80 μg/mL L-NAME (standard diet/L-NAME 80 group, n=8). The animals in the cholesterol diet group were divided...
were cut open longitudinally, and the intimal surface was rinsed in 50% ethanol for 1 minute and immersed in formalin. The total and lesioned areas were measured by planimetry of photographic images, and the degree of atherosclerosis was expressed as a percentage of the luminal surface area covered by lesions. After morphometric analysis, aortic segments were stained with hematoxylin-eosin and examined by light microscopy by a pathologist for the presence of atherosclerosis.

**Drugs**

The pharmacological agents used were L-NE bitartrate, Ach chloride, L-NAME (all from Sigma Chemical Co), and (9,11)-(11,12)-dideoxa-9a,11α-dimethylmethano-11,12-methano-13,14-dihydro-13-aza-14-oxo-15-cyclopentyl-16,17,18,19,20-pentanor-15-epi-TXA2 (ONO-3708; Ono Pharmaceutical Co).

**Vascular Reactivity Studies**

The thoracic aortic tissue was placed in cold Krebs-Henseleit solution of the following composition (millimoles per liter): NaCl 118, KCl 4.7, CaCl2 2.55, MgSO4 1.8, KH2PO4 24.88, glucose 11.1, and CaNa2EDTA 0.026. The fat and connective tissue were removed, and thoracic aortas were cut with scissors into rings 5 mm long. Two stainless steel wires were inserted into the vascular lumen and one wire was connected to a force transducer (model TB-612T, Nihon Kohden). The rings were suspended in an organ chamber containing 30 mL Krebs-Henseleit solution that was bubbled with a 95% O2/5% CO2 mixture and maintained at 37°C. The rings were incubated for 90 minutes for equilibration at a resting tension of 3 g with buffer exchanges every 15 minutes during this period. The rings were manipulated carefully to avoid producing unnecessary tension or damage to the endothelium.

Aortic rings were subsequently contracted with norepinephrine (NE, 10^-7 mol/L), and after contraction reached a plateau, they were relaxed with a cumulative dose of Ach (10^-9 mol/L to 10^-4 mol/L). A TXA2/PGH2 receptor antagonist, ONO-3708 (10^-6 mol/L), was added to the bath solution 15 minutes before NE-induced contraction. The rings were precontracted with 10^-7 mol/L NE (A), Ach only contracting the rings (Fig 1C).

**Typical Responses of Aortic Rings to Ach**

Ach at 10^-6 mol/L maximally relaxed the aortic rings precontracted with 10^-7 mol/L NE. At higher concentrations, Ach induced contractions (Fig 1A). On the other hand, Ach contracted but barely relaxed the rings without an endothelium (Fig 1B). Similarly, after pretreatment with 10^-4 mol/L L-NAME, Ach only contracted the rings (Fig 1C).

**Effect of Hypercholesterolemia**

**Biochemical and Physiological Measurements**

At the start of the experiment, body weight was similar in the standard diet/normal water group and cholesterol diet/normal water groups. Weight gain in the cholesterol diet/normal water group at 8 and 12 weeks was significantly lower than that in the standard diet/normal water group. Rabbits in the cholesterol diet/normal water group had higher total cholesterol and high-density lipoprotein cholesterol levels at 8 and 12 weeks compared with their counterparts in the standard diet/normal water group (Table 1).
TABLE 1. Body Weights and Lipid Values in Each Rabbit Group

<table>
<thead>
<tr>
<th></th>
<th>8 Weeks</th>
<th>12 Weeks</th>
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</thead>
<tbody>
<tr>
<td>Standard diet/normal water group</td>
<td></td>
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</tr>
<tr>
<td>Baseline BW, g</td>
<td>2581 ± 48 (6)</td>
<td>2586 ± 59 (5)</td>
</tr>
<tr>
<td>Increase in BW, g</td>
<td>1051 ± 96 (8)</td>
<td>1248 ± 147 (5)</td>
</tr>
<tr>
<td>Total chol, mg/dL</td>
<td>27 ± 4 (8)</td>
<td>20 ± 3 (5)</td>
</tr>
<tr>
<td>HDL-chol, mg/dL</td>
<td>14 ± 3 (8)</td>
<td>12 ± 2 (5)</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>59 ± 9 (8)</td>
<td>54 ± 30 (5)</td>
</tr>
<tr>
<td>Cholesterol diet/normal water group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline BW, g</td>
<td>2755 ± 84 (8)</td>
<td>2675 ± 68 (12)</td>
</tr>
<tr>
<td>Increase in BW, g</td>
<td>605 ± 137 (6)*</td>
<td>868 ± 82 (12)*</td>
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<tr>
<td>Total chol, mg/dL</td>
<td>1143 ± 198 (6)*</td>
<td>1232 ± 128 (12)*</td>
</tr>
<tr>
<td>HDL-chol, mg/dL</td>
<td>35 ± 5 (6)*</td>
<td>26 ± 6 (12)</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>47 ± 10 (6)</td>
<td>38 ± 14 (12)</td>
</tr>
<tr>
<td>Standard diet/L-NAME 80 group</td>
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</tr>
<tr>
<td>Baseline BW, g</td>
<td>2505 ± 41 (4)</td>
<td>2540 ± 31 (4)</td>
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<tr>
<td>Increase in BW, g</td>
<td>735 ± 124 (4)</td>
<td>1043 ± 52 (4)</td>
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<tr>
<td>Total chol, mg/dL</td>
<td>26 ± 4 (4)</td>
<td>25 ± 6 (4)</td>
</tr>
<tr>
<td>HDL-chol, mg/dL</td>
<td>16 ± 4 (4)</td>
<td>18 ± 4 (4)</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>30 ± 6 (4)</td>
<td>33 ± 15 (4)</td>
</tr>
<tr>
<td>Cholesterol diet/L-NAME 80 group</td>
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<td></td>
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<tr>
<td>Baseline BW, g</td>
<td>2680 ± 93 (6)</td>
<td>2648 ± 63 (5)</td>
</tr>
<tr>
<td>Increase in BW, g</td>
<td>507 ± 106 (6)</td>
<td>726 ± 164 (5)</td>
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<tr>
<td>Total chol, mg/dL</td>
<td>1632 ± 335 (6)*</td>
<td>1649 ± 396 (5)*</td>
</tr>
<tr>
<td>HDL-chol, mg/dL</td>
<td>36 ± 4 (6)</td>
<td>22 ± 2 (5)</td>
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<tr>
<td>TG, mg/dL</td>
<td>30 ± 9 (6)</td>
<td>72 ± 30 (5)</td>
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<tr>
<td>Cholesterol diet/L-NAME 160 group</td>
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<tr>
<td>Baseline BW, g</td>
<td>2585 ± 60 (6)</td>
<td>2514 ± 40 (6)</td>
</tr>
<tr>
<td>Increase in BW, g</td>
<td>380 ± 80 (6)</td>
<td>644 ± 90 (6)</td>
</tr>
<tr>
<td>Total chol, mg/dL</td>
<td>2161 ± 279 (6)**</td>
<td>1903 ± 110 (6)**</td>
</tr>
<tr>
<td>HDL-chol, mg/dL</td>
<td>36 ± 4 (6)</td>
<td>29 ± 4 (6)</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>51 ± 24 (6)</td>
<td>62 ± 24 (6)</td>
</tr>
</tbody>
</table>

BW indicates body weight; total chol, total cholesteroi; HDL-chol, high-density lipoprotein cholesterol; TG, triglycerides; L-NAME, Nω-nitro-L-arginine methyl ester; and (n), number of animals examined.

*P < .05 vs standard diet/normal water group.
**P < .05 vs cholesterol diet/normal water group.

Vascular Reactivity Studies

Maximal tension induced by 10^-7 mol/L NE in the cholesterol diet/normal water group was similar to that in the standard diet/normal water group: 0.91 ± 0.08 versus 0.90 ± 0.07 g at 8 weeks and 0.89 ± 0.10 versus 0.87 ± 0.15 g at 12 weeks. Aortic rings in the cholesterol diet/normal water group showed significantly less maximal relaxation in response to Ach than those in the standard diet/normal water group: 61.0 ± 4.5% versus 81.5 ± 2.7% at 8 weeks (P < .01) and 45.4 ± 12.5% versus 87.2 ± 4.0% at 12 weeks (P < .05) (Fig 2). Pretreatment with the TXA2/PGH2 receptor antagonist ONO-3708 did not reverse this impaired endothelium-dependent relaxation (Fig 3).

Effect of Orally Administered L-NAME

Biochemical and Physiological Measurements

Body weight gain during the experiment as well as initial body weight in the cholesterol diet/L-NAME group was similar to that in the cholesterol diet/normal water group. The cholesterol diet/L-NAME 80 group had a total cholesterol level similar to that in the cholesterol diet/normal water group, although the total cholesterol level in the cholesterol diet/L-NAME 160 group was significantly higher than that in the cholesterol diet/normal water group (Table 1). L-NAME in drinking water did not affect systemic blood pressure (Table 2). Rabbits in each group daily consumed a similar amount of drinking water.

Vascular Reactivity Studies

Maximal tensions induced by 10^-7 mol/L NE in aortic rings from the standard diet/normal water and the standard diet/L-NAME 80 groups were 0.90 ± 0.07 and 1.02 ± 0.05 g, respectively, at 8 weeks and 0.87 ± 0.15 and...
Table 2. Hemodynamic Variables for Each Rabbit Group

<table>
<thead>
<tr>
<th>Rabbit Group</th>
<th>8 Weeks</th>
<th>12 Weeks</th>
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<tbody>
<tr>
<td>Standard diet/normal water group</td>
<td></td>
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</tr>
<tr>
<td>SBP, mm Hg</td>
<td>104.2±1.0 (6)</td>
<td>101.4±1.7 (5)</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>80.3±1.2 (6)</td>
<td>76.3±1.5 (5)</td>
</tr>
<tr>
<td>Cholesterol diet/normal water group</td>
<td></td>
<td></td>
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<tr>
<td>SBP, mm Hg</td>
<td>105.8±4.2 (6)</td>
<td>98.5±1.5 (12)</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>82.4±4.8 (6)</td>
<td>74.5±3.5 (12)</td>
</tr>
<tr>
<td>Cholesterol diet/L-NAME 80 group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>102.4±2.1 (4)</td>
<td>102.5±3.4 (4)</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>82.5±2.6 (4)</td>
<td>75.4±4.6 (4)</td>
</tr>
<tr>
<td>Cholesterol diet/L-NAME 160 group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>99.2±3.0 (6)</td>
<td>101.8±6.2 (5)</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>77.7±2.6 (6)</td>
<td>82.4±3.5 (5)</td>
</tr>
<tr>
<td>Cholesterol diet/L-NAME 160 group</td>
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<td></td>
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<tr>
<td>SBP, mm Hg</td>
<td>95.6±2.4 (6)</td>
<td>100.3±3.7 (6)</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>79.2±3.5 (6)</td>
<td>83.4±4.4 (6)</td>
</tr>
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</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; L-NAME, N^o-nitro-L-arginine methyl ester; and (n), number of animals examined.

0.98±0.05 g, respectively, at 12 weeks. Corresponding values in the cholesterol diet/normal water, cholesterol diet/L-NAME 80, and cholesterol diet/L-NAME 160 groups were 0.91±0.08, 0.93±0.08, and 0.96±0.07 g, respectively, at 8 weeks and 0.89±0.10, 0.85±0.15, and 0.90±0.09 g respectively, at 12 weeks. Oral administration of L-NAME did not affect NE-induced maximal tension of aortic rings from both normocholesterolemic and hypercholesterolemic rabbits.

Aortic rings from the standard diet/L-NAME 80 group showed more impaired maximal relaxation to Ach compared with those from the standard diet/normal water group: 58.7±2.3% versus 81.5±2.7% at 8 weeks (P<.01) and 50.9±4.2% versus 87.2±4.0% at 12 weeks (P<.01) (Fig 4). The cholesterol diet/L-NAME 80 group had markedly less maximal relaxation of aortic rings in response to Ach than did the cholesterol diet/normal water group: 30.3±7.3% versus 61.0±4.5% at 8 weeks (P<.05) and 71.9±9.1% versus 45.4±12.5% at 12 weeks (P<.05) (Fig 5). This was also true for the cholesterol diet/L-NAME 160 group: 27.4±8.6% versus 61.0±4.5% at 8 weeks (P<.05) and 18.1±1.6% versus 45.4±12.5% at 12 weeks (P<.05) (Fig 5). L-NAME in drinking water impaired maximal aortic ring relaxation to Ach in hypercholesterolemic as well as normocholesterolemic rabbits.

Morphometric and Histological Analyses

Thoracic aortas from normocholesterolemic rabbits had no evidence of atheromatous lesions. The percentages of fatty plaque areas in thoracic aortas from rabbits in the cholesterol diet/L-NAME 80 and cholesterol diet/L-NAME 160 groups were 15.2±4.4% and 25.5±7.2%, respectively, at 8 weeks and 74.7±9.7% and 57.3±4.9%, respectively, at 12 weeks. Corresponding values in the cholesterol diet/normal water group were 4.7±2.0% at 8 weeks and 23.8±6.9% at 12 weeks (Fig 6). Oral administration of L-NAME for 8 and 12 weeks significantly promoted atherosclerotic lesions in hypercholesterolemic rabbits. Histological study of the aortas demonstrated typical atherosclerotic changes both in hypercholesterolemic rabbits given L-NAME and in those not given L-NAME. However, atherosclerotic lesions were more advanced in rabbits given L-NAME.

Discussion

Effect of PGH2 on Vascular Responses in Atherosclerotic Rabbits

The vascular endothelium produces EDCFs as well as EDRF and controls vascular smooth muscle tone, and imbalance between these factors seems to augment vascular tone in various pathological states. In the rat thoracic aorta, vascular relaxation occurs through EDHF production in response to low concentrations of Ach. The cholesterol diet/L-NAME 80 and cholesterol diet/L-NAME 160 groups had markedly less maximal relaxation of aortic rings in response to Ach than did the cholesterol diet/normal water group: 30.3±7.3% versus 61.0±4.5% at 8 weeks (P<.05) and 71.9±9.1% versus 45.4±12.5% at 12 weeks (P<.05) (Fig 5). This was also true for the cholesterol diet/L-NAME 160 group: 27.4±8.6% versus 61.0±4.5% at 8 weeks (P<.05) and 18.1±1.6% versus 45.4±12.5% at 12 weeks (P<.05) (Fig 5). L-NAME in drinking water impaired maximal aortic ring relaxation to Ach in hypercholesterolemic as well as normocholesterolemic rabbits.
Ach, but high concentrations of Ach cause vascular contraction. We have revealed that this contractile response is due to endothelium-derived PGH2 in old WKYs,9,10 SHR,9-11 and diabetic rats.12 In this study, we examined whether similar changes occurred in the hypercholesterolemic rabbit thoracic aorta.

Thoracic aortas of normocholesterolemic rabbits showed relaxation and contractile responses to Ach similar to those of rats. Vascular relaxation was inhibited by pretreatment with L-NAME, which indicated that this relaxation response was attributable to EDRF. However, contraction also was noted in vascular specimens from which the endothelium had been removed, suggesting that this contractile response was not mediated through the action of the endothelium but rather by a direct action of Ach on vascular smooth muscle.

In aortic rings from hypercholesterolemic rabbits, vascular relaxation in response to Ach was weaker than that of rings from normocholesterolemic rabbits. No significant difference in the direct contractile effects of Ach was observed in endothelium-denuded aortas in normocholesterolemic and hypercholesterolemic rabbits. Therefore, reduction in the relaxation response to Ach seems to be due to the impaired endothelium-dependent relaxation. This impaired endothelium-dependent relaxation has been reported in atherosclerotic rabbits.21-23 Several factors, such as an abnormality in synthesizing and/or releasing EDRF,24 enhanced release of inactivated forms of NO and its related compounds,25 deactivation of NO by free radicals,26-28 and impaired cholinoeceptor activity,29 have been proposed as possible causes, but the exact mechanism of this impaired relaxation remains unknown. We hypothesize that PGH2 as an EDCF also contributes to attenuation of the endothelium-dependent relaxation in atherosclerotic rabbits, as occurs in hypertensive and diabetic rats. However, ONO-3708, a PGH2/TXA2 receptor antagonist, had no effect on the attenuated relaxation response, which indicated that participation of PGH2 in the process was unlikely. There were no differences in vascular relaxation responses to sodium nitroprusside and the response to Ach in specimens pretreated with L-NAME between normocholesterolemic and hypercholesterolemic rabbits (data not shown). Therefore, our findings indicate that reduced production of endothelium-derived NO rather than stimulated EDCF synthesis seems to attenuate the endothelium-dependent vascular response and may lead to enhanced formation of atherosclerotic lesions in hypercholesterolemic rabbits. Changes in vascular responses may vary with various species and pathological states.

Effect of Long-term Administration of L-NAME on Atherosclerosis Progression

Numerous factors have been implicated in the initiation and development of atherosclerosis, including blood pressure, plasma cholesterol levels, lipoprotein oxidation, vascular smooth muscle tone, and platelet aggregation. Therapies that attenuate atherogenesis usually do so by affecting one or more of these factors. It has been reported that calcium channel blockers,30 TXA2 inhibitors,31 angiotensin-converting enzyme inhibitors,32 and probucol, an antioxidant,33,34 inhibit atherosclerosis progression.

Recently, the relationship between NO and atherosclerosis has attracted attention. NO is known to be present not only in the vascular endothelium but also in the central nervous system,35,36 peripheral nerves,37 kidney,38 pancreas,39 and macrophages.40,41 Endothelium-derived NO, which acts as an EDRF, and macrophase-derived NO coexist in atherosclerotic lesions, but their functional relationship has not been clarified.

To evaluate the roles of NO in the pathogenesis of atherosclerosis, we examined the effects of long-term administration of L-NAME, which inhibits NO synthetase, on atherosclerosis progression. Long-term oral administration of L-NAME significantly diminished Ach-induced relaxation of aortic rings from normocholesterolemic rabbits. The relaxation response was almost completely abolished either by removal of the endothelium or by ex vivo pretreatment with L-NAME (data not shown), which indicates that reduced synthesis of endothelium-derived NO may be responsible for attenuation of the relaxation response. No atherosclerotic lesions were found in specimens from normocholesterolemic rabbits. In hypercholesterolemic rabbits, endothelium-dependent relaxation was more impaired and the atherosclerotic lesion area was markedly enlarged by administration of L-NAME at concentrations of both 80 μg/mL and 160 μg/mL in drinking water.

We measured two variables that may be responsible for atherosclerosis progression, one of which is the serum cholesterol level. It is especially important to note that L-NAME at 80 μg/mL appears to have a small but not statistically significant effect on cholesterol level and that at the higher concentration (160 μg/mL) the effect is significant. However, it should be noted that L-NAME at both concentrations promoted atherosclerosis. Therefore, the effect of L-NAME on atherosclerosis is reasonably considered to be derived from its inhibition of NO synthesis but not from its hypercholesterolemic effect, if L-NAME has such an effect at all. The other variable that we measured was blood pressure. Administration of an NO synthetase inhibitor has been reported to elevate blood pressure in rats42-44 and rabbits,45,46 but in our study, long-term oral administration of L-NAME did not raise blood pressure despite a
reduced endothelium-derived NO production, as indicated by impaired relaxation of aortic rings in response to Ach. Differences in species or routes of administration could explain the discrepancy between our results and those of others. In previous studies, rabbits were administered L-NAME as a bolus intravenous injection,9,16 whereas ours used an oral long-term administration of L-NAME dissolved in drinking water. Our method may maintain lower but more stable serum L-NAME levels. Even though a precise underlying mechanism has not been clarified, our results indicate that L-NAME can exacerbate atherosclerosis without raising blood pressure.

The mechanism by which L-NAME exerts its atherogenic effects in hypercholesterolemic rabbits is still unclear. Endothelium-derived NO production was obviously suppressed by oral administration of L-NAME in our study, because the aortic rings showed an attenuated relaxation response to Ach. NO not only is a potent vasodilator but also has an inhibitory effect on platelet adhesion and aggregation.17-19 NO has also been reported to inhibit monocyte adherence to cultured endothelial cells20 and to control endothelial permeability.48,49 Our results suggest that L-NAME inhibits the antiatherogenic effects of NO and thus promotes atherosclerosis. A recent finding supports our results by showing that long-term oral administration of L-arginine, the metabolic precursor for NO, reverses the attenuated endothelium-dependent relaxation of aortic rings in response to Ach and prevents atherosclerosis development in hypercholesterolemic rabbits.50 Thus, reduced production of endothelium-derived NO is atherogenic and increased production antiatherogenic, indicating that NO may play a crucial role in preventing atherosclerosis. However, NO derived from cells other than endothelial cells may contribute to the antiatherogenic effect.1 It is also possible that L-NAME may directly (not through inhibition of NO synthesis) act on monocyte adherence or infiltration, platelet reactivity, or other key processes in atherogenesis, but there has been no evidence to support these hypotheses. Our findings indicate that long-term oral administration of L-NAME enhances atherosclerosis progression in the aorta of hypercholesterolemic rabbits by inhibiting NO production.

Conclusions
The endothelium-derived vascular relaxation response is diminished in the atherosclerotic rabbit thoracic aorta. Unlike its role in hypertensive and diabetic rats, PGI2 as an EDCF is not responsible for this impaired endothelium-dependent relaxation. Rather, our findings suggest that this phenomenon is attributable to the reduction in NO synthesis or release. In addition, reduced production of NO in the aortic wall, which has also been observed in animals after long-term oral administration of L-NAME, further enhanced atherosclerosis progression, thus creating a vicious circle of pathophysiological events.

Acknowledgment
We thank Dr Masafumi Ito (Department of Pathology, Nagoya University Hospital) for his helpful assistance in the morphometric analysis and histological examination of atherosclerotic rabbit aortas.

References


Long-term inhibition of NO synthesis promotes atherosclerosis in the hypercholesterolemic rabbit thoracic aorta. PGH2 does not contribute to impaired endothelium-dependent relaxation.

K Naruse, K Shimizu, M Muramatsu, Y Toki, Y Miyazaki, K Okumura, H Hashimoto and T Ito

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