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 endothelial-dependent contracting factor, or the disturbed production of endothelium-derived relaxing factor, impairs endothelial-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 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.

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

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Adherence, platelet aggregation, and vascular smooth muscle cell proliferation. 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 N₂-nitro-L-arginine methyl ester (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

Sudan red at 37°C for 120 minutes. Next, the tissues were examined for the presence of fatty plaques. The specimens were inserted into the vascular lumen and one wire was connected to a force transducer (model TB-612T, Nihonkoh Den). 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} \text{ mol/L}), and after contraction reached a plateau, they were relaxed with a cumulative dose of Ach (10^{-9} \text{ mol/L to 10^{-4} mol/L}). A TXA2/PGH2 receptor antagonist, ONO-3708 (10^{-6} \text{ mol/L}), was added to the bath 15 minutes before NE-induced contraction. 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).

into three sets: those drinking untreated water (cholesterol diet/normal water group, n=18) and those drinking water containing either 80 or 160 \mu g/mL L-NAME (cholesterol diet/L-NAME 80 group, n=11; cholesterol diet/L-NAME 160 group, n=12). L-NAME was administered throughout the course of the study.

After 8 and 12 weeks of dietary treatment, the central ear arteries of conscious restrained rabbits were cannulated by percutaneous puncture with a 24G cannula for measurement of intra-arterial blood pressure and blood sampling. Blood pressure was measured through the arterial cannula after the animal was allowed to rest quietly for at least 15 minutes. Then the rabbits were anesthetized with pentobarbital sodium (30 mg/kg) given via the marginal ear vein.Thoracic aortas were harvested for studies of vascular reactivity and morphometry. These protocols were approved by the animal ethics committee of Nagoya University.

Vascular Reactivity Studies

The thoracic aorta tissue was placed in cold Krebs-Henseleit solution of the following composition (millimoles per liter): NaCl 118, KCl 4.7, CaCl2 2.55, MgSO4 1.18, KH2PO4 24.88, glucose 11.1, and Ca,Na2EDTA 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, Nihonkoh Den). 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.

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Morphometric and Histological Analyses

To investigate the effect of long-term administration of L-NAME on atherosclerosis development, morphometric and histological analyses of fatty plaque areas were performed. Thoracic aortas (from the left subclavian artery to the diaphragm) including the rings used for vascular reactivity studies were cut open longitudinally, and the intimal surface was examined for the presence of fatty plaques. The specimens were rinsed in 50% ethanol for 1 minute and immersed in Sudan red at 37°C for 120 minutes. Next, the tissues were transferred to 50% ethanol for 5 minutes, washed in running distilled water for 10 minutes, and stored in 10% buffered 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).

Fig 1. Representative records of acetylcholine (Ach)-induced responses of thoracic aortas from rabbits in the standard diet/normal water group. A and B, Rings with (A) and without (B) endothelium were contracted with 10^{-7} \text{ mol/L norepinephrine (NE)}, and Ach was added cumulatively (10^{-9} \text{ to 10^{-4} mol/L}). C, 10^{-2} \text{ mol/L L-arginine methylester (L-NAME)} was added 15 minutes before NE-induced contraction.

Drugs were dissolved in distilled water and concentrations expressed as the final concentration in the bath solution.

Statistical Analysis

Results were expressed as mean±SEM. For statistical analysis, Student’s t tests for paired or unpaired observations were used. Values of $P<.05$ were considered significant.

Typical Responses of Aortic Rings to Ach

Ach at 10^{-6} \text{ mol/L} maximally relaxed the aortic rings precontracted with 10^{-7} \text{ 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} \text{ 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 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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<tbody>
<tr>
<td><strong>Standard diet/normal water group</strong></td>
<td></td>
<td></td>
</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>
</tbody>
</table>

| **Cholesterol diet/normal water group** |                 |                 |
| Baseline BW, g       | 2755±84 (8)     | 2675±68 (12)    |
| Increase in BW, g    | 605±137 (6)*    | 868±82 (12)*    |
| Total chol, mg/dL    | 1143±198 (6)*   | 1232±128 (12)*  |
| HDL-chol, mg/dL      | 35±5 (6)*       | 26±6 (12)       |
| TG, mg/dL            | 47±10 (6)       | 38±14 (12)      |

| **Standard diet/L-NAME 80 group** |                 |                 |
| Baseline BW, g       | 2505±41 (4)     | 2540±31 (4)     |
| Increase in BW, g    | 735±124 (4)     | 1043±52 (4)     |
| Total chol, mg/dL    | 26±4 (4)        | 25±4 (4)        |
| HDL-chol, mg/dL      | 16±4 (4)        | 18±4 (4)        |
| TG, mg/dL            | 30±6 (4)        | 33±15 (4)       |

| **Cholesterol diet/L-NAME 80 group** |                 |                 |
| Baseline BW, g       | 2680±93 (6)     | 2648±63 (5)     |
| Increase in BW, g    | 507±106 (6)     | 726±164 (5)     |
| Total chol, mg/dL    | 1632±335 (6)*   | 1649±396 (5)*   |
| HDL-chol, mg/dL      | 36±4 (6)        | 22±2 (5)        |
| TG, mg/dL            | 30±9 (6)        | 72±30 (5)       |

| **Cholesterol diet/L-NAME 160 group** |                 |                 |
| Baseline BW, g       | 2585±60 (6)     | 2514±40 (6)     |
| Increase in BW, g    | 380±80 (6)      | 644±90 (6)      |
| Total chol, mg/dL    | 2161±279 (6)*   | 1903±110 (6)**  |
| HDL-chol, mg/dL      | 36±4 (6)        | 29±4 (6)        |
| TG, mg/dL            | 51±24 (6)       | 62±24 (6)       |

BW indicates body weight; total chol, total cholesterol; HDL-chol, high-density lipoprotein cholesterol; TG, triglycerides; L-NAME, N\(^\text{-nitro-l-arginine methylester; and (n), number of animals examined.}

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

\(9\text{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 \((P<0.01)\) and 45.4±12.5% versus 87.2±4.0% at 12 weeks \((P<0.05)\) (Fig 2). Pretreatment with the TXA\(_2/P\)GH\(_2\) receptor antagonist ONO-3708 did not reverse this impaired endothelium-dependent relaxation (Fig 3).

Fig 2. Acetylcholine (Ach)-induced relaxations in rabbit aortic rings precontracted with \(10^{-7}\) mol/L norepinephrine (NE) in standard diet/normal water and cholesterol diet/normal water groups at 8 (left) and 12 (right) weeks of dietary intervention. Ach \((10^{-9} \text{ to } 10^{-4}\text{ mol/L})\) was applied by cumulative addition to aortic rings precontracted with \(10^{-7}\text{ mol/L NE. Results are shown as mean±SEM.} *P<.05, **P<.01\) compared with standard diet/normal water group. n, number of rabbits.

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 1.02±0.05 g, respectively, 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 \((P<0.01)\) and 45.4±12.5% versus 87.2±4.0% at 12 weeks \((P<0.05)\) (Fig 2). Pretreatment with the TXA\(_2/P\)GH\(_2\) receptor antagonist ONO-3708 did not reverse this impaired endothelium-dependent relaxation (Fig 3).

Fig 3. Effect of ONO-3708 \((10^{-4}\text{ mol/L})\) on acetylcholine (Ach)-induced relaxations in aortic rings from rabbits in cholesterol diet/normal water group at 8 (left) and 12 (right) weeks of dietary intervention. Two aortic rings, one for control and the other for ONO-3708 pretreatment, from the same rabbit were compared. Ach \((10^{-9}\text{ to } 10^{-4}\text{ mol/L})\) was applied by cumulative addition to aortic rings precontracted with \(10^{-7}\text{ mol/L NE. Results shown are mean±SEM.} n, number of rabbits.
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</td>
<td>101.4±1.7</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>80.3±1.2</td>
<td>76.3±1.5</td>
</tr>
<tr>
<td>Cholesterol diet/normal water group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>105.8±4.2</td>
<td>98.5±1.5</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>82.4±4.8</td>
<td>74.5±3.5</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</td>
<td>102.5±3.4</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>82.5±2.6</td>
<td>75.4±4.6</td>
</tr>
<tr>
<td>Cholesterol diet/L-NAME 80 group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>99.2±3.0</td>
<td>101.8±6.2</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>77.7±2.6</td>
<td>82.4±3.5</td>
</tr>
<tr>
<td>Cholesterol diet/L-NAME 160 group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>95.6±2.4</td>
<td>100.3±3.7</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>79.2±3.5</td>
<td>83.4±4.4</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; L-NAME, N\(^{-}\)nitro-L-arginine methylester; 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±1.1% 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 (P<.01) (Fig 6). The cholesterol diet/L-NAME 80 group had significantly more advanced atherosclerotic lesions than the cholesterol diet/L-NAME 160 group and the cholesterol diet/normal water group. 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 PGH\(_2\) on Vascular Responses in Atherosclerotic Rabbits

The vascular endothelium produces EDCFs\(^{1-2}\) as well as EDRF\(^{2-4}\) 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 EDRF production in response to low concentrations of Ach. However, the cholesterol diet/L-NAME groups showed more impaired maximal relaxation to Ach compared with the control groups. This was also true for the cholesterol diet/L-NAME 160 group. The results shown are mean±SEM. **P<.01 compared with standard diet/normal water group. n, number of rabbits.
Atherosclerosis Progression

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, TXA2 inhibitors, angiotensin-converting enzyme inhibitors, and probucol, an antioxidant, 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, peripheral nerves, kidney, pancreas, and macrophages. 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 synthesis, 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 inactivation 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 rats and rabbits, but in our study, long-term oral administration of L-NAME did not raise blood pressure despite a
The 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, 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. NO has also been reported to inhibit monocyte adherence to cultured endothelial cells and to control endothelial permeability. 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. 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. 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 atherosclerosis, 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, PGH 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

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16. Myers PR, Minor RL Jr, Guerra R Jr, Bates JN, Harrison DG. Atherosclerosis impairs endothelium-dependent vascular mechanism has not been clarified, our results indicate that L-NAME can exacerbate atherosclerosis without raising blood pressure.

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

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