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EXPERIMENTAL STUDIES

Amelioration of Severity of Myocardial Injury by a Nitric Oxide Donor in Rabbits Fed a Cholesterol-Rich Diet

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Objectives. **This study compared the effect of a nitric oxide donor on limiting the size of infarct resulting from myocardial ischemia–reperfusion between atherosclerotic and nonatherosclerotic models.**

Background. **Endothelial-derived relaxation in coronary arteries affected by ischemia is substantially impaired after reperfusion, and this impairment may exacerbate the myocardial ischemia–reperfusion injury. In animals with experimental atherosclerosis, release of endothelial-derived relaxing factor is also decreased, and the propagation of myocardial infarction could be exacerbated.**

Methods. **We examined the extent of myocardial injury induced by ischemia (30 min) and reperfusion (48 hr) in rabbits fed a cholesterol-rich (1%) or normal diet for 10 weeks. We also evaluated the effect of a nitric oxide donor (*S*-nitroso-*N*-acetylpenicillamine [SNAP]), a nitric oxide precursor (L-arginine) or a degradation product of SNAP (*N*-acetylpenicillamine) on infarct size in these models.**

Results. **Severity of myocardial injury was significantly exacerbated in cholesterol-fed rabbits (75.2 ± 4.4% [mean ± SEM]) compared with that in non–cholesterol-fed rabbits (53.2 ± 5.2%). This exacerbation was prevented by treatment with SNAP (50.2 ± 6.4%) but not with L-arginine (70.5 ± 6.0%) or *N*-acetylpenicillamine (70.4 ± 4.8%) in cholesterol-fed rabbits. However, SNAP did not limit infarct size in non–cholesterol-fed rabbits (60.8 ± 4.2%). The rate-pressure product was similar during the course of the experiment in all the groups.**

Conclusions. **Myocardial damage induced by ischemia–reperfusion was significantly exacerbated in rabbits fed a long-term cholesterol-rich diet but was effectively reversed by treatment with a nitric oxide donor. However, this agent did not limit infarct size in normal rabbits. Thus, a nitric oxide donor reduces myocardial infarct size in atherosclerotic but not in nonatherosclerotic rabbits.**

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Endothelium-dependent relaxation of the aorta and coronary arteries is impaired in rabbits with experimental atherosclerosis induced by a cholesterol-rich diet (1,2). Coronary vascular reserve is also reduced in Watanabe heritable hyperlipidemic rabbits (3). Endothelium-derived relaxing factor is nitric oxide or a nitroso compound that generates nitric oxide (4,5). There is considerable evidence that hypercholesterolemia and atherosclerosis are associated with increased oxygen-derived free radical production, which is thought to degrade nitric oxide and to account for deficiencies in endothelial-dependent relaxation (6–8). Pathophysiologic concentrations of low density lipoprotein (LDL) may also inhibit endothelium-dependent relaxation (9). In humans, hypercholesterolemia impairs endothelium-dependent dilation of the coronary microcirculation. This impairment can be corrected by short-term administration of L-arginine (10), a precursor of nitric oxide, or by the reduction of serum cholesterol (11). Release of endothelium-derived relaxing factor in coronary arteries affected by ischemia is substantially decreased after reperfusion, even in nonatherosclerotic animals (12–14), and this decrease may exacerbate the myocardial ischemia-reperfusion injury as a result of increased leukocyte and platelet aggregation as well as leukocyte adherence to the coronary endothelium, migration into the extravascular space and plugging (15,16). Administration of nitric oxide donors or L-arginine can minimize the myocardial injury associated with ischemia-reperfusion (17,18). However, the role of nitric oxide in ischemia-reperfusion injury is still controversial (19,20). Few studies have shown an increased severity of acute myocardial ischemia in chronically atherosclerotic animals (21), although infarct size was not evaluated in that study. Acute hypercholesterolemia also increases the severity of myocardial ischemia by a mechanism dependent on the no-reflow phenomenon (22–24). Recently, we reported that inhibition of nitric oxide synthesis exhibits the augmentation of myocardial infarct size in rabbits (25). In this study, we compared the extent of myocardial ischemia-reperfusion injury between cholesterol-fed and control rabbits. To investigate differences in the role of nitric oxide

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in the propagation of myocardial injury between the two models, we also examined the effect of a stable nitric oxide donor (*S*-nitroso-*N*-acetylpenicillamine [SNAP]) (26), or L-arginine on infarct size. These agents were administered immediately before coronary artery occlusion because a decrease in nitric oxide synthesis may occur during ischemia-reperfusion in experimentally atherosclerotic rabbits.

Methods

Housing and feeding. Adult male Japanese white rabbits (n = 40) were fed a diet of standard laboratory food supplemented with 1% cholesterol for 10 weeks. A second group of rabbits (n = 21) was fed standard laboratory food without added cholesterol. Water was available ad lib. All animals were housed under identical conditions (Nihon Bioresearch, Inc., Hashima, Japan). Arterial blood samples anticoagulated with heparin (50 U/ml) were collected immediately before the 10-week feeding period and again at the end of the 10 weeks for the determination of plasma cholesterol concentration.

Experimental protocol. After 10 weeks on their respective diets, the rabbits were anesthetized with sodium pentobarbital injected into the marginal ear vein (30 mg/kg), intubated and then ventilated with the use of a small animal respirator (model 683, Harvard Apparatus). The left femoral artery was used for the continuous measurement of arterial blood pressure with a pressure transducer (TP-300T, Nihon Kohden, Tokyo, Japan) and heart rate, and the left femoral vein was used for drug administration and blood sampling. Lead II of the electrocardiogram (ECG) was monitored throughout the study. Hemodynamic variables were continuously recorded (model WT-645G recorder, Nihon Kohden). Left thoracotomy was performed and the heart was exposed. A silk thread was passed around a branch of the left circumflex coronary artery with a tapered needle, and the ends of the thread were passed through a small vinyl tube. The branch was occluded by pulling the snare, which was fixed by clamping the tube with a mosquito clamp. Occlusion of the coronary artery caused cyanosis and akinesis in the ischemic region and a marked ST segment increase on the ECG. After 30 min of coronary occlusion, the snare was released; reperfusion was indicated by a change in color of the ventricular surface. The surgical wounds were repaired 30 min after reperfusion, and the rabbits were returned to their cages for recovery. An aseptic surgical technique was observed throughout and benzylpenicillin (30,000 U/kg) was injected intramuscularly for infection prophylaxis. Forty-eight hours after operation, each rabbit was injected intravenously with 1000 U of heparin and administered an overdose of pentobarbital. The heart was then removed for postmortem analysis.

The cholesterol-fed rabbits were divided into four groups treated with 1) SNAP (15 μg/kg body weight as a bolus plus 5 μg/kg/min), 2) L-arginine (30 mg/kg as a bolus plus 10 mg/kg/min), 3) a degradation product of SNAP, *N*-acetylpenicillamine (15 μg/kg as a bolus plus 5 μg/kg/min), or 4) vehicle (isotonic saline). The non–cholesterol-fed rabbits were treated with either SNAP or isotonic saline. SNAP, L-arginine, *N*-acetylpenicillamine and isotonic saline were infused into a femoral vein catheter with a syringe pump for 60 min beginning immediately before coronary occlusion and ending 30 min after reperfusion.

Although some investigators have administered a high dose of SNAP continuously (>10 μg/kg per min) (27,28), such doses induced a significant decrease in systemic blood pressure. The dose of SNAP we used (5 μg/kg per min) also induced a slight decrease (~10%) in systemic blood pressure, but it had no significant effect on the rate–pressure product in either the noncholesterol-fed or cholesterol-fed rabbits in a preliminary study. The dose of L-arginine (10 mg/kg per min) used in our study is similar to that previously administered to hypercholesterolemic rabbits (29,30) and to that used in a myocardial ischemia–reperfusion model (31). This dose of L-arginine did not exert a significant effect on the rate–pressure product in our previous study (25). The SNAP was obtained from Dojindo Laboratories (Kumamoto, Japan), L-arginine from Wako (Osaka, Japan) and *N*-acetylpenicillamine from Sigma Chemical.

Measurement of infarct size. Hearts were perfused with saline through the aorta to wash out residual blood. Evans blue dye (2%) was introduced after reocclusion of the left coronary branch to estimate the area perfused by the occluded artery (ischemic region). The left ventricle was cut parallel to the apex base axis into six pieces, which were then incubated with 1% triphenyltetrazolium chloride at 37°C for 10 min to stain the noninfarcted region. The ischemic, infarct and nonischemic regions were separated with scissors and weighed. The area at risk and infarct size were defined as ischemic region/left ventricular and infarct region/ischemic region mass ratios, respectively, and were expressed as percentages, as previously described (32,33).

Assessment of leukocyte accumulation. Myeloperoxidase activity was assessed by the method of Bradley et al. (34), but modified slightly, as described previously (35). Myocardial tissue was taken from the nonischemic and ischemic regions of the heart, frozen rapidly in liquid nitrogen, pulverized and suspended in 50 mmol/liter potassium phosphate buffer (pH 6.0). After centrifugation at 40,000 × *g* for 15 min, the supernatant was assayed spectrophotometrically for myeloperoxidase activity; the change in absorbance at 460 nm was measured with dianisidine–hydrogen peroxide as the substrate. One unit of myeloperoxidase activity was defined as that capable of degrading 1 μmol peroxide/min at 30°C. Myocardial leukotriene B_4 content was determined by the method of Fradin et al. (36), with slight modification. In brief, the myocardial tissues suspended in 50 mmol/liter potassium phosphate buffer (pH 6.0) were centrifuged at 3,000 × *g* for 5 min at 4°C, and acetic acid was added to the supernatants until a concentration of 0.1 mol/liter was reached. With a Bond Elut C2 column (Varian) leukotriene B_4 was extracted from the acidified supernatants with ethyl acetate. After the extract was

Table 1. Mortality Rate in Experimental Groups

Group	No. of Rabbits				Mortality Rate (%)
	Ventricular Fibrillation				
	Occlusion	Reperfusion	Late Death*	Survived	
Non–cholesterol-fed rabbits					
Vehicle (n = 12)	1	0	1	10	17
SNAP (n = 9)	0	0	1	8	11
Cholesterol-fed rabbits					
Vehicle (n = 13)	1	0	2	10	23
SNAP (n = 10)	1	0	1	8	20
L-Arginine (n = 10)	1	0	2	7	30
AP (n = 7)	1	0	1	5	28

*Death on the first postoperative day. No significant difference was seen in mortality among all six groups. AP = *N*-acetylpenicillamine; SNAP = *S*-nitroso-*N*-acetylpenicillamine.

evaporated to dryness, the concentration of leukotriene B_4 was determined with a specific enzyme immunoassay (Cayman).

Measurement of atherosclerotic plaque area. The surface area of atherosclerotic lesions of the descending thoracic aorta in cholesterol-fed rabbits was determined by removing the thoracic aorta from the left subclavian artery to the diaphragm. Adventitial tissue was dissected free and the remaining blood was rinsed away. Each aorta was bisected longitudinally and fixed in 10% buffered formalin. The specimens were immersed in Sudan red stain at room temperature for 15 min and then transferred to 80% ethanol for 20 min. Measurements of lesion surface area and total aortic surface area were made by planimetry of photographic images.

Statistical analysis. Results are expressed as mean value ± SE. Differences in infarct size, myeloperoxidase activity, leukotriene B_4 content, atherosclerotic area of thoracic aorta among groups and hemodynamic changes over time were determined by analysis of variance. A p value < 0.05 was considered significant. When multiple comparisons were performed, Tukey's test was used to determine the p value required for statistical significance. Analysis of covariance was used to compare the regression lines between the area at risk and infarct size among the groups.

Results

Mortality and characteristics. Five rabbits died of ventricular fibrillation during coronary occlusion and eight died on the first day after the operation, probably because of arrhythmia or heart failure. Although the mortality rates for the four groups of cholesterol-fed rabbits appeared higher than those for the other two groups, the difference was not statistically significant (chi-square test) (Table 1). The 48 surviving rabbits were subjected to various analyses. The change in body mass and the plasma total cholesterol concentration before the 10-week feeding period showed no significant differences among the six groups (Table 2). Among the four groups of cholesterol-fed rabbits, there were no significant differences in the plasma concentration of total cholesterol and in the area of atherosclerotic plaque of the thoracic aorta at 10 weeks.

Hemodynamic variables. The systolic (Fig. 1A) and diastolic (Fig. 1B) blood pressure after coronary occlusion in the two SNAP-treated groups was lower than that in the corresponding vehicle-treated groups, but the differences were not statistically significant. The heart rate (Fig. 1C) was slightly higher in non–cholesterol-fed rabbits than in cholesterol-fed rabbits. However, the rate-pressure product, an index of

Table 2. Characteristics of Experimental Groups (mean ± SE)

Group	Body Mass (kg)		Total Plasma Cholesterol (mg/dl)		Area of Atherosclerotic Plaque of Thoracic Aorta (%)
	Before	After	Before	After	
Non–cholesterol-fed rabbits					
Vehicle (n = 10)	2.7 ± 0.1	2.9 ± 0.1*	23 ± 2	19 ± 2	—
SNAP (n = 8)	2.6 ± 0.1	3.0 ± 0.1*	24 ± 3	21 ± 3	—
Cholesterol-fed rabbits					
Vehicle (n = 10)	2.7 ± 0.1	2.9 ± 0.1*	25 ± 3	2,232 ± 219†	11.1 ± 2.0
SNAP (n = 8)	2.5 ± 0.1	3.0 ± 0.1*	29 ± 3	2,220 ± 213†	14.2 ± 2.9
L-Arginine (n = 7)	2.6 ± 0.1	2.9 ± 0.1*	26 ± 2	1,880 ± 154†	12.0 ± 3.2
AP (n = 5)	2.6 ± 0.1	2.9 ± 0.1*	27 ± 3	2,044 ± 207†	10.6 ± 2.5

*$p < 0.05$, †$p < 0.001$ versus before cholesterol feeding. Body mass and total plasma cholesterol concentration were determined immediately before and after the 10-week feeding period.

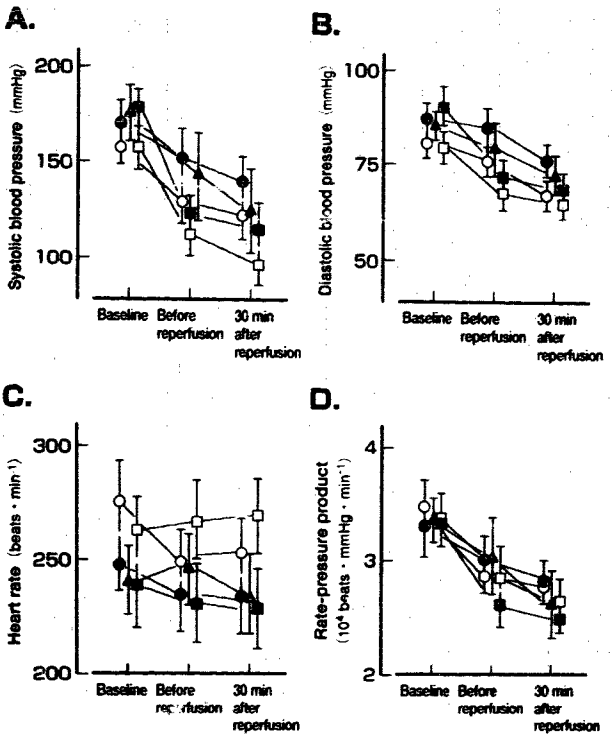

Figure 1. Hemodynamic variables measured at baseline, immediately before reperfusion and 30 min after reperfusion for cholesterol-fed (**solid symbols**) and non–cholesterol-fed (**open symbols**) rabbits treated with vehicle (**circles**), *S*-nitroso-*N*-acetylpenicillamine (**squares**) or L-arginine (**triangles**). The rate–pressure product is systolic blood pressure multiplied by heart rate. Data are expressed as mean ± SE. Multiple comparisons of variables among the experimental groups were performed by analysis of variance (Tukey's test). Although the hemodynamic data for cholesterol-fed rabbits treated with *N*-acetylpenicillamine were not shown, there were no significant differences in hemodynamic variables among all six groups.

myocardial oxygen consumption, was similar in all of the experimental groups (Fig. 1D). Although not shown, changes in the hemodynamic data in cholesterol-fed rabbits treated with *N*-acetylpenicillamine were not significantly different from the other five groups.

Infarct size. We expressed the area of the ischemic heart at risk and the amount of necrotic cardiac tissue as a percentage of the total left ventricular mass and of the area at risk, respectively. There were no significant differences in the area at risk (Fig. 2A). There were also no significant differences in the degree of ST segment elevation before reperfusion and 30 min after reperfusion among all six groups (data not shown). However, the infarct size was significantly higher ($p < 0.05$) in vehicle-treated cholesterol-fed rabbits (75.2 ± 4.4%) than in vehicle-treated non–cholesterol-fed rabbits (53.2 ± 5.2%), indicating that the myocardial damage induced by ischemia-reperfusion was significantly exacerbated in cholesterol-fed rabbits (Fig. 2B). The infarct size in non–cholesterol-fed rabbits treated with SNAP (60.8 ± 4.2%) did not differ significantly from that in the corresponding vehicle group. However, the infarct size in cholesterol-fed rabbits treated with SNAP (50.2 ± 6.4%) was significantly ($p < 0.05$) smaller than that in the corresponding vehicle group, although there was no significant difference in infarct size between the vehicle and L-arginine (70.5 ± 6.0%) or *N*-acetylpenicillamine (70.4 ± 4.8%) groups of cholesterol-fed rabbits.

The absolute infarct size significantly correlated ($p < 0.01$) with the size of the area at risk in all groups: 1) non–cholesterol-fed: vehicle, $y = 0.90x - 0.69$, $r = 0.872$; SNAP, $y = 0.82x - 0.36$, $r = 0.904$; 2) cholesterol-fed: vehicle, $y = 1.09x - 0.66$, $r = 0.898$; SNAP, $y = 1.08x - 0.99$, $r = 0.898$; L-arginine, $y = 1.17x - 0.87$, $r = 0.935$; *N*-acetylpenicillamine, $y = 1.02x - 0.39$, $r = 0.962$. Furthermore, the regression line for the vehicle group of cholesterol-fed rabbits was significantly different ($p < 0.05$) from that for the vehicle group of non–cholesterol-fed rabbits and the SNAP group of cholesterol-fed rabbits (Fig. 3). The regression lines did not differ significantly between the two SNAP groups or between the vehicle and SNAP groups of non–cholesterol-fed rabbits. These observations indicate that SNAP reduced the infarct size in the cholesterol-fed rabbits but not in the non–cholesterol-fed rabbits, regardless of the size of the area at risk.

Leukocyte accumulation. To determine the effects of SNAP on the accumulation of leukocytes into the ischemic myocardium, we examined myocardial myeloperoxidase activity and leukotriene B_4 content in nonischemic and ischemic regions. Although myeloperoxidase activity and leukotriene B_4 content in the nonischemic myocardium did not vary among

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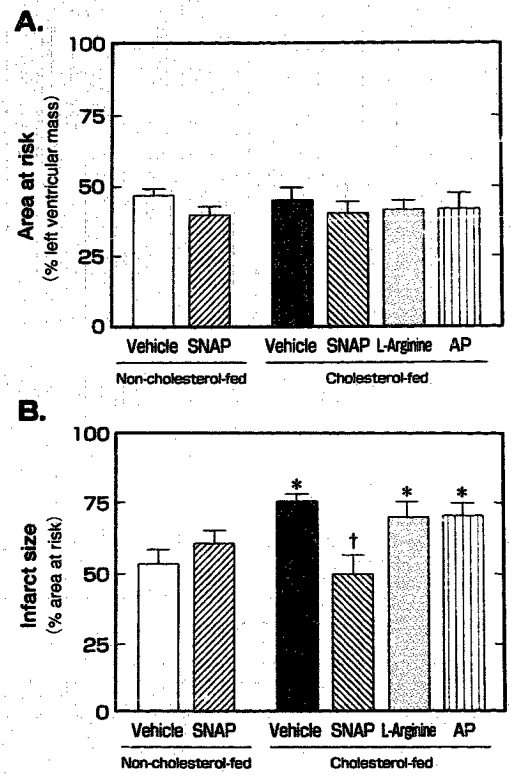

Figure 2. Area at risk **(A)** and infarct size **(B)** for cholesterol-fed and non-cholesterol-fed rabbits treated with vehicle, *S*-nitroso-*N*-acetylpenicillamine (SNAP), L-arginine or *N*-acetylpenicillamine (AP). Data are expressed as mean value ± SE. *$p < 0.05$ versus vehicle-treated non-cholesterol-fed rabbits. †$p < 0.05$ versus vehicle-treated cholesterol-fed rabbits (analysis of variance with Scheffé test).

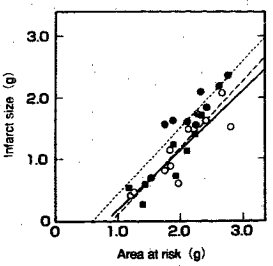

Figure 3. Linear regression analysis of the area at risk versus infarct size. A linear relation was apparent for each group. The regression line for the vehicle-treated group of cholesterol-fed rabbits **(solid circles)** differed significantly from those for the vehicle-treated group of non-cholesterol-fed rabbits **(open circles)** and the *S*-nitroso-*N*-acetylpenicillamine-treated group of cholesterol-fed rabbits **(solid squares)**. $p < 0.05$, analysis of covariance.

the groups (data not shown), myeloperoxidase activity and leukotriene B_4 content in the ischemic myocardium of the vehicle and L-arginine groups of cholesterol-fed rabbits were significantly increased ($p < 0.05$) relative to the corresponding values for the vehicle and SNAP groups of non-cholesterol-fed rabbits and the SNAP group of cholesterol-fed rabbits (Fig. 4).

Discussion

Hypercholesterolemia and nitric oxide. In both humans and rabbits, coronary vascular reserve is markedly attenuated in the hypercholesterolemic state, possibly because increased oxygen-derived free radical production by the arterial wall results in degradation of nitric oxide (6–8); a decreased availability of L-arginine to synthesize nitric oxide has been proposed in the coronary vasculature (29,30); or nitric oxide is inactivated by native and oxidized LDL accumulated in the vascular wall (37,38). An increase in the serum concentration of an endogenous inhibitor of nitric oxide synthesis has been demonstrated in hypercholesterolemic rabbits (39). In our preliminary study, we observed that the plasma NO_3^- plus NO_2^- concentration was significantly lower in our cholesterol-fed rabbits than in our non-cholesterol-fed rabbits, although constitutive nitric oxide synthase in aorta was more potently expressed in cholesterol-fed rabbits as assessed by immunohistochemistry. This finding supports the hypothesis that an impairment of endothelium-dependent vasodilation in atherosclerotic vessels may not be due to a decrease in constitutive nitric oxide synthase expression (40). Measurement of hemodynamic variables revealed no difference in the sensitivity of the vascular bed to SNAP between normal and atherosclerotic rabbits, although Moncada et al. (41) demonstrated that removal of basal nitric oxide release results in increased supersensitivity of vascular tissue to nitrovasodilators.

Nitric oxide in atherosclerotic rabbits. The increased severity of injury induced by acute myocardial ischemia in experimental atherosclerotic rabbits may result from the reduction in nitric oxide synthesis or the increased inactivation of nitric oxide produced in the coronary circulation and consequent exacerbation of the no-reflow phenomenon. A decrease in the amounts of active nitric oxide augments leukocyte adherence to the endothelium of resistance vessels as well as aggregation of leukocytes and platelets. This sequence of events causes microcirculatory disorders of the ischemic coronary bed. Thromboxane A_2 generation and platelet aggregation have been shown to be increased in the hypercholesterolemic state (42–45). Reactive oxygen species production (45) and vasoconstrictor effects of products from activated leukocytes (46) are known to be enhanced in hypercholesterolemia. SNAP, by serving as a donor of nitric oxide, may inhibit the aggregation of leukocytes and platelets as well as leukocyte adherence to the coronary endothelium provoked by reduction in active nitric oxide production (16–18), thus resulting in a decrease in leukocyte infiltration and limitation of infarct size

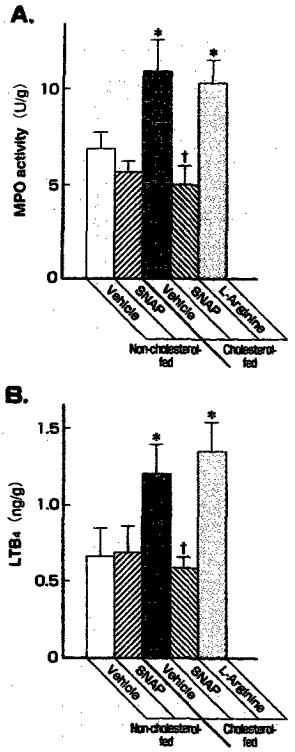

Figure 4. Myeloperoxidase (MPO) activity (**A**) and leukotriene B_4 (LTB_4) content (**B**) in the experimental groups. Values (mean ± SE) represent levels in ischemic myocardium after subtraction of levels in nonischemic myocardium. *$p < 0.05$ versus the vehicle-treated group of non-cholesterol-fed rabbits. †$p < 0.05$ versus the vehicle-treated group of cholesterol-fed rabbits (analysis of variance with Scheffé test). SNAP = *S*-nitroso-*N*-acetylpenicillamine.

in cholesterol-fed rabbits. Our data on myeloperoxidase activity and leukotriene B_4 content in the myocardium support this conclusion. It is possible that nitric oxide is acting to scavenge superoxide anion, which is increased in both coronary arteries and myocardium in atherosclerotic animals (47), leading to limitation of infarct size. Because rabbits were used in our experiment owing to the extremely minimal native coronary collateral supply in this species (48), administration of SNAP is unlikely to reduce myocardial injury by increasing the extent of collateralization. It is not clear why exogenous L-arginine did not exhibit beneficial effects similar to those of SNAP on infarct size in the atherosclerotic rabbits; exogenous L-arginine has been shown to normalize the endothelium-dependent vasodilation in chronically hypercholesterolemic rabbits (29,30). However, our results are compatible with the notion that cholesterol feeding prevents incorporation of nitric oxide into a more potent nitrosylated compound or accelerates the intracellular or extracellular degradation of endothelium-derived relaxing factor (7,8,49). In an in vitro study, L-arginine proved to have no effect on improving endothelial dysfunction in atherosclerotic aorta (50).

Nitric oxide in nonatherosclerotic rabbits. Use of SNAP showed no effect on infarct size in non-cholesterol-fed rabbits. This observation appears inconsistent with those of previous studies showing that nitric oxide donors and L-arginine reduce myocardial ischemia–reperfusion injury (17,18). An increase in the endogenous production of nitric oxide during ischemia–reperfusion may be enough to suppress the propagation of myocardial infarction in nonatherosclerotic rabbits. In fact, we recently reported that inhibition of nitric oxide synthesis by treatment with N^G-nitro-L-arginine methyl ester exacerbates myocardial ischemia-reperfusion injury in rabbits (25). However, excess amounts of exogenous nitric oxide added during ischemia–reperfusion may fail to limit infarct size further, possibly because interaction of nitric oxide with superoxide anion generates peroxynitrite and hydroxyl radical (51), which may be cytotoxic in excess. We also reported that treatment with L-arginine did not show an infarct-limiting effect in nonatherosclerotic rabbits (25). A similar observation has been described for the intestinal vascular injury apparent in the acute phase of endotoxin shock: The physiologic formation of nitric oxide by constitutive nitric oxide synthase is important in protecting against tissue injury, but the inappropriate release of nitric oxide by the administration of high doses of SNAP can result in extensive tissue injury (52–54). Our results suggest a complex role for nitric oxide in protecting against myocardial injury. The effect of SNAP on infarct size may differ depending on the dose, species and time of administration. We did not examine the effect of a larger dose of SNAP on infarct size because continuous infusion of such a dose of SNAP (15 μg/kg per min) caused a significant reduction in systemic blood pressure.

Study limitations. Exacerbation of myocardial injury in cholesterol-fed rabbits may be related to the presence of coronary artery stenosis. In the four cholesterol-fed groups we found no difference in the extent of area of atherosclerotic plaque of the thoracic aorta and no significant stenotic lesions of epicardial coronary arteries, although our evaluation of stenotic coronary artery lesions was incomplete because of the measurement of infarct size. Osborne et al. (21) reported that there is a consistent degree of stenosis in cholesterol-fed rabbits, comprising 50% to 75% of the coronary vessel lumen of small arteries. However, the infarct size even in rabbits with acute hypercholesterolemia (cholesterol fed for 4 days) was significantly higher than that in control rabbits, and this exacerbation was also prevented by treatment with SNAP (unpublished observations). Because the effects of hypercholesterolemia on the vessels do not develop with very short-term hypercholesterolemia and coronary flow reserve depends mainly on nitric oxide production in resistance vessels, the significance of stenotic coronary arteries themselves in the propagation of myocardial injury may be small. Nitric oxide donors have been shown to attenuate the endothelial dysfunction observed in reperfused coronary arteries of nonathero-

sclerotic animals ex vivo (17,55), but it remains to be seen whether they can attenuate the endothelial dysfunction in those of atherosclerotic animals. It would be better to examine this issue in an in vivo state, although we cannot do these experiments in a rabbit model. Recently, Zweier et al. (56) directly showed an increased production of nitric oxide in myocardial tissue during ischemia, although we did not measure nitric oxide production in the coronary circulation during ischemia-reperfusion. The possibility that SNAP is effective by another mechanism independent of nitric oxide release in protecting against myocardial injury in atherosclerotic rabbits cannot be excluded.

Conclusions. We showed that 1) myocardial ischemia–reperfusion injury is exacerbated in rabbits fed a long term cholesterol-rich diet compared with that in non–cholesterol-fed rabbits; 2) the augmentation of myocardial injury can be reversed by administration of the nitric oxide donor, SNAP, but not the nitric oxide precursor L-arginine; and 3) treatment with SNAP exerts no effect on infarct size in non–cholesterol-fed rabbits. In chronically hypercholesterolemic rabbits, the adequate amounts of nitric oxide endogenously produced during ischemia-reperfusion would be reduced, leading to propagation of myocardial infarction, although the excess amounts of nitric oxide may be deleterious in myocardial injury. In the clinical setting, active nitric oxide production in the coronary circulation of most patients who have undergone a myocardial infarction would be severely attenuated, similar to that in chronically cholesterol-fed rabbits. Although the atherosclerotic state in this experimental model is not the same as that in humans, one may need to examine the effect of an agent on limiting infarct size in chronically atherosclerotic animals before trying to test it in humans. Some agents may reduce myocardial infarct size in atherosclerotic but not in normal animals.

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