Long-term Cholesterol Feeding Alters the Reactivity of Primate Coronary Microvessels to Platelet Products

James E. Quillen, Frank W. Sellke, Mark L. Armstrong, and David G. Harrison

Hypercholesterolemia impairs endothelium-dependent relaxations to several platelet-derived substances in large vessels. The effect of hypercholesterolemia on the response of coronary microvessels to platelet products and thrombin is less well defined. Three groups of cynomolgus monkeys were studied: normal (n=6), short-term hypercholesterolemic (8-11 weeks, n=5), and long-term hypercholesterolemic (18-80 months, n=6). Responses of coronary microvessels, 100-200 μm in diameter, to thrombin (0.1-10 units/ml) and the platelet products ADP (1 nM-100 μM), serotonin (1 nM-100 μM), and the thromboxane A₂ analogue U46619 were studied using an in vitro microvessel imaging apparatus. Vessels were studied after precontraction with thromboxane A₂ analogue U46619 to evaluate both relaxations and constrictions to each agent. Concentrations of U46619 to attain precontraction were much lower for the long-term group (16 ±19 nM) as compared with the control and short-term hypercholesterolemic groups (689 ±48 and 664 ±63 nM, respectively, p<0.01). Relaxations of long-term hypercholesterolemic vessels to ADP tended to be less than those of either control or short-term hypercholesterolemic vessels. Thrombin, when added to normal or short-term hypercholesterolemic vessels, caused identical relaxations but paradoxically caused constrictions in microvessels of long-term hypercholesterolemic monkeys (55 ±17% of KC1 contraction, p<0.001 vs. other groups). Peak constrictions to serotonin were markedly enhanced in the long-term hypercholesterolemic group (59 ±7% of maximal KC1 responses) compared with control and short-term hypercholesterolemic responses (28±8% and 32±5%, respectively, both p<0.05 vs. long-term hypercholesterolemia). In contrast, relaxations to adenosine and nitroprusside were identical in all groups. Long-term cholesterol feeding markedly altered the responses of the coronary microcirculation to several platelet products. These findings may have important implications regarding the regulation of myocardial perfusion in long-term but not short-term exposure to elevated cholesterol levels. (Arteriosclerosis and Thrombosis 1991;11:639–644)

Human atherosclerosis leads to multiple acute coronary syndromes including vasospastic angina, unstable angina, and acute myocardial infarction. Several lines of evidence support a role for platelets and thrombin deposition in the development of these acute processes. Aspirin, which attenuates platelet thromboxane A₂ production by the inhibition of cyclooxygenase, reduces myocardial infarction and death in patients with unstable angina.1-3 Ticlopidine, which alters platelet adhesive properties and possibly interferes with platelet activation,4 reduces the risk of fatal and nonfatal myocardial infarction in patients with unstable angina.5 Additionally, the platelet product thromboxane A₂, measured as the breakdown product thromboxane B₂, is found in high concentrations in coronary sinus blood from patients with unstable angina,6 and elevated levels of 2,3-dinor-thromboxane B₂, a breakdown product of thromboxane A₂, are found in the urine of these same patients.7 Platelet products also contribute to cyclic flow reductions at the site of chronic artificial coronary stenoses.8-16 These findings clearly advocate a role for platelets and platelet products in acute coronary syndromes.
Large-vessel atherosclerosis importantly alters conduit vascular reactivity to platelet products and thrombin by eliciting augmented constrictions to serotonin and blunted relaxations to thrombin. This has been largely attributed to endothelial dysfunction. It is now clear that chronic hypercholesterolemia not only alters endothelium-derived relaxing factor of large vessels but also has marked effects on the coronary microcirculation.

In the absence of severe proximal coronary stenosis, coronary flow is regulated by the coronary microcirculation. Hypercholesterolemia produces conduit-vessel atherosclerosis; however, the coronary microcirculation demonstrates no gross anatomic changes with cholesterol feeding. Endothelium-dependent relaxations to acetylcholine, bradykinin, and calcium ionophore A23187 are impaired in coronary microvessels after 18 months of cholesterol feeding in cynomolgus monkeys. The time required to develop endothelial dysfunction in the coronary microcirculation has, however, not been addressed in previous studies. Further, the effect of chronic hypercholesterolemia on vascular responses to agonists released from acutely formed thrombi has not been elucidated. Responses to such agents are particularly important because platelet aggregation and thrombosis may release products that adversely affect the vascular tone of the coronary microcirculation.

The purpose of this study was to directly evaluate the short-term and long-term effects of cholesterol feeding on coronary microvascular reactivity to platelet products and thrombin in primates.

Methods

Adult male cynomolgus monkeys were studied. Six monkeys were fed a commercial laboratory diet (Purina Monkey Chow, Ralston-Purina, Richmond, Ind.). Short-term and long-term cholesterol-fed monkeys were fed an atherogenic diet of 0.7% cholesterol and 40% fat for 8-12 weeks and 18-80 months, respectively. At intervals of 3-4 months and immediately before they were killed, the monkeys were sedated with ketamine hydrochloride (10 mg/kg body wt i.m.), and venous blood samples were drawn for measurement of cholesterol and triglyceride levels. The monkeys were killed and were placed in cold Krebs' buffer (118.3 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 11.1 mM glucose, pH 7.4). Epicardial vessels were washed three times with Krebs' buffer and were placed in an isolated Plexiglas organ chamber, and both ends were cannulated with glass micropipettes measuring 50-100 μm in diameter and were secured to the micropipettes with 10-0 nylon monofilament suture. Oxygenated (95% O₂, 5% CO₂) Krebs' buffer warmed to 37°C was continuously circulated through the organ chamber.

The optimal distending pressure for contraction to KCl was found to occur at 20 mm Hg. The vessels were therefore pressurized to 20 mm Hg in a no-flow state using a manometer filled with Krebs' buffer. A pressure transducer measured distending pressures at a sidearm immediately adjacent to one of the micropipettes. By use of a split-screen microscope connected to a video camera, the vessel image was projected onto a television monitor. A video electronic dimension analyzer was used to measure luminal diameter. Measurements were recorded with a strip chart recorder. After at least 1 hour of equilibration, the microvessels were studied after preconstriction with U46619 (a stable thromboxane A₂ analogue) to allow an assessment of relaxation as well as constriction to each agent. Preconstriction of 20-30% of the resting baseline diameter was attained before serotonin dose-response curves, as serotonin has inherent constrictor effects, whereas 30-60% of resting baseline diameter was attained for all other dose-response curves. One to three dose-response curves were obtained for each vessel. Vessels were washed three times with Krebs' buffer and allowed to equilibrate at least 15 minutes between interventions. Increasing concentrations of ADP, serotonin, thrombin, adenosine, and nitroprusside were studied after preconstriction.

Drugs

ADP, serotonin, thrombin, adenosine, and nitroprusside were obtained from Sigma Chemical Co., St. Louis, Mo. U46619 was obtained from The Upjohn Co., Kalamazoo, Mich. All drugs were dissolved in water. All drug solutions were made fresh each day with the exception of U46619, which was stored at -20°C as a stock solution.

Data Analysis

Relaxation responses of the coronary microvessels were expressed as percent relaxation from their preconstricted diameters. Constriction responses were expressed as percent of maximal KCl constriction. Mean responses at each dose of each drug were compared by analysis of variance. Whenever significance was indicated, Scheffe's F test for multiple comparisons was used for between-group comparisons. Values were expressed as mean±SEM. Significance was assumed if p<0.05.

Results

The duration of the atherogenic diets in the short-term group of monkeys ranged from 8 to 11 weeks, averaging 9.5 weeks. The long-term group diets ranged from 18 to 80 months, averaging 35 months. Serum triglyceride levels were similar between groups, averaging 29±5, 19±8, and 30±10 mg/dl in the control, short-term, and long-term groups, respectively (p=NS). Serum cholesterol levels in the control group were 111±14 mg/dl, whereas the short-term and long-term group values were elevated to 814±81 and 680±50 mg/dl, respectively (p<0.01 vs. control).
Cholesterol Alters Reactivity of Coronary Microvessels

Microvessel Characteristics

Coronary microvessels from the left anterior descending distribution were between 100–200 μm in diameter, averaging 158±5, 165±5, and 166±4 μm in the control, short-term, and long-term cholesterol-fed monkeys, respectively (p=NS).

Responses to Thromboxane A₂ Analogue U46619

Preconstriction to 30–60% of resting diameter was attained by the addition of thromboxane A₂ analogue U46619. Diameters after constriction with U46619 for the control, short-term, and long-term groups were 89±5, 93±5, and 94±6 μm, respectively (p=NS). The mean concentration of U46619 required to attain preconstriction was 689±48, 664±63, and 16±19 nM (p<0.01 vs. control) for the control, short-term, and long-term groups, respectively (Figure 1).

Responses to Platelet Products and Thrombin

In coronary microvessels, relaxations to ADP were mildly impaired by long-term hypercholesterolemia as compared with control and short-term hypercholesterolemia (Figure 2).

Thrombin, when added to control or short-term hypercholesterolemic preconstricted vessels, caused identical relaxations but paradoxically caused constrictions in the long-term hypercholesterolemic vessels of 55±17% of the KCl value (p<0.001, Figure 3).

In control and short-term hypercholesterolemia, serotonin caused mild constriction (28±8% and 32±5%, respectively, Figure 4). Long-term hypercholesterolemia produced marked constriction (59±7% of maximal KCl constriction, p<0.05 vs. control and short-term groups).

Responses to Adenosine and Nitroprusside

Adenosine produced complete relaxation with identical dose–response curves in control, short-term, and long-term hypercholesterolemic groups (Figure 5). Nitroprusside similarly produced complete and equal relaxations in all groups (Figure 6).

Discussion

It is now well established that chronic hypercholesterolemia dramatically impairs endothelium-dependent vascular relaxation of large vessels, including monkey iliac arteries, rabbit aortas, pig coronary arteries, and human coronary arteries. Because the coronary microcirculation does not develop overt atherosclerosis, it was reasonable to assume that endothelial cell function would not be altered within this segment of the vasculature. It is now evident, however, that chronic hypercholesterol-
Arteriosclerosis and Thrombosis Vol 11, No 3 May/June 1991

Control, n=6
Short term, n=5
Long term, n=6

* p<0.05 long vs. short and control

Thrombin (units/ml)

Figure 3. Semilogarithmic line plot of average responses (percent relaxation or constriction) from preconstricted tension of coronary microvessels of control (-○-, n=6), short-term (-■-, n=5), and long-term (-△-, n=6) hypercholesterolemic monkeys to thrombin (units/ml). Values are mean±SEM. *p<0.05 for long-term vs. short-term and control.

Figure 4. Line plot of average constrictions (percent) of coronary microvessels from control (-○-, n=6), short-term (-■-, n=5), long-term (-△-, n=6) hypercholesterolemic monkeys to serotonin (log concentration). Values are mean±SEM. *p<0.05 for long-term vs. control.

Figure 5. Line plot of average relaxations (percent) of coronary microvessels of control (-○-, n=5), short-term (-■-, n=5), and long-term (-△-, n=6) hypercholesterolemic monkeys to adenosine (log concentration). Values are mean±SEM.

Elevated plasma concentrations of thrombin can impair endothelium-dependent vascular relaxation within the microcirculation. The present study adds to the previous literature by showing that potent vasoactive products involved in the thrombotic process produce strikingly different responses in coronary microvessels from primates fed a high-cholesterol diet for 18 months compared with vessels from normal animals. The present study also shows that shorter-term exposure to hypercholesterolemia had minimal effects on microvascular endothelium-dependent relaxations to these agents. In the present study, relaxations to adenosine and nitroprusside were not altered by either short-term or long-term cholesterol feeding. Because these compounds produce relaxation by direct actions on vascular smooth muscle, it is reasonable to assume the primary alteration of vasomotion observed in the long-term group was primarily related to an abnor-
mality of endothelial function. The endothelial dysfunction likely accounts for the blunt relaxation to ADP and the high doses of serotonin, as well as a large portion of the responses to thrombin. The present findings do not exclude other mechanisms underlying the enhanced constrictions to serotonin and the thromboxane analogue U46619. It is conceivable that chronic hypercholesterolemia induces the expression of larger quantity or a different subtype of receptors for these agonists on vascular smooth muscle. This possibility is strengthened by the recent observation that proliferating smooth muscle cells increase synthesis of the serotonin receptor and the relatively recent recognition that numerous serotonin-receptor subtypes may exist on vascular smooth muscle.29 Similarly, increased sensitivity (approximately 50-fold) to the thromboxane analogue U46619 may be due to alterations of the thromboxane receptor on vascular smooth muscle resulting from chronic hypercholesterolemia.

Previous studies have suggested that the duration of hypercholesterolemia necessary for the development of endothelial cell dysfunction may be quite brief. In cholesterol-fed rabbits, abnormal endothelium-dependent vascular relaxation may occur after only 4 weeks of cholesterol feeding.24 Moreover, acute exposure to low density lipoprotein can alter endothelium-dependent vascular relaxation of isolated rings of rabbit aorta.30 The present findings suggest that these prior observations either may not apply to the microcirculation or may be species dependent.

There is substantial evidence to support a role for acute thrombosis in the genesis of acute ischemic syndromes.31-37 It is quite likely that vasoactive substances released from coronary thrombi or from platelets adherent to the intimal surface may importantly modulate vascular tone, producing episodic coronary spasm. Additionally, platelets exposed to hypercholesterolemia may be hyperaggregable and generate more thromboxane A₂ than do normal platelets.38,39 These effects of platelet products and thrombi have largely been considered in relation to the larger epicardial coronary arteries.28,40 Based on the present findings, it is interesting to speculate that substances such as thromboxane A₂, serotonin, and thrombin released from large-vessel thrombi may be delivered to the distal vasculature and produce constriction of the coronary microcirculation. This microvascular constriction would be substantially augmented in the setting of chronic hypercholesterolemia and might contribute to a worsening of myocardial ischemia.

References


KEY WORDS • hypercholesterolemia • atherosclerosis • coronary microvessels • platelets • thrombin • serotonin • thromboxane A2 • adenosine diphosphate
Long-term cholesterol feeding alters the reactivity of primate coronary microvessels to platelet products.

J E Quillen, F W Sellke, M L Armstrong and D G Harrison

doi: 10.1161/01.ATV.11.3.639

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/11/3/639