In Vivo Temperature Heterogeneity of Atherosclerotic Plaques Is Determined by Plaque Composition

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Background—Temperature heterogeneity of atherosclerotic plaques has been associated with macrophage accumulation in ex vivo studies. We investigated in vivo whether modifying the cell composition of rabbit atherosclerotic plaques by dietary cholesterol lowering can influence temperature heterogeneity.

Methods and Results—Twenty New Zealand rabbits were randomized to either a normal (n=10) or cholesterol-rich (0.3%) diet (n=10) for 6 months. Thereafter, intravascular ultrasound and intravascular catheter-based thermography of the surface of aortic arch and descending aorta were performed in all animals. Ten control and 5 hypercholesterolemic rabbits were euthanized, and their aortas were analyzed histologically. The 5 remaining rabbits received a normal diet for 3 months and underwent repeat ultrasound and thermography before euthanasia followed by histology. Ex vivo temperature was measured in 3 additional rabbits at 6 months to correlate local temperature with local plaque composition. In control animals, plaque formation and temperature heterogeneity were absent. In hypercholesterolemic rabbits, plaque formation was prominent in the thoracic aorta. Plaques were composed of fibromuscular tissue and contained, underneath endothelial cells, an accumulation of foam cells of macrophage origin. Temperature heterogeneity was markedly elevated and increased with plaque thickness. Importantly, after 3 months of cholesterol lowering, plaque thickness remained unchanged, but temperature heterogeneity was significantly decreased. This paralleled plaque histology, which showed a marked loss of macrophages. The ex vivo experiments demonstrated the relation between local temperature and local total macrophage mass.

Conclusions—In vivo temperature heterogeneity of rabbit atherosclerotic plaques is determined by plaque composition. In vivo thermography may have important clinical implications in the assessment of plaque composition. (Circulation. 2002;105:1596-1601.)

Key Words: atherosclerosis • catheters • hypercholesterolemia • lipids • plaque

atheromatous plaque rupture is thought to play an important role in the pathogenesis of acute ischemic syndromes.1,2 Atherosclerotic plaques contain a central necrotic core separated from the vascular lumen by a fibrous cap composed of smooth muscle cells and collagen. Plaque rupture predominantly occurs at the shoulder of the plaque, where the fibrous cap is thin and contains an accumulation of activated macrophages underneath it.3-4

Previous studies have shown that there is marked temperature heterogeneity in human carotid5 and coronary6 atherosclerotic plaques. The heterogeneity in coronary arteries was found to be larger in patients with unstable angina and acute myocardial infarction as opposed to normal coronary arteries and patients with stable angina.6 Unstable plaques are associated with an increased macrophage accumulation as opposed to stable plaques.7 Therefore, the determination of temperature heterogeneity may have a pivotal role in predicting plaque composition and could largely contribute to the differentiation between stable and unstable plaques. Indeed, temperature heterogeneity may be associated with sites of an increased concentration of macrophages and other inflammatory cells that may play a crucial role in heat release within atherosclerotic plaques. Atherosclerotic plaques in the descending thoracic aorta of hypercholesterolemic rabbits contain a collagen-rich matrix, a paucity of smooth muscle cells, and an abundance of macrophages; these characteristics are reminiscent of some histological aspects of the human unstable atherosclerotic plaque.8-9 Moreover, dietary cholesterol lowering in rabbits results in dramatic changes in plaque composition by a
reduction of macrophages and an increase of collagen formation without changing plaque thickness,\textsuperscript{8,9} resembling some histological features of the human stable atherosclerotic plaque.

The aim of the present in vivo study in rabbits was to investigate whether there is an association between the temperature heterogeneity of atherosclerotic plaques and plaque composition. Temperature heterogeneity in the plaques was studied before and after dietary cholesterol lowering. To assess whether local temperature is related to local plaque morphology, additional ex vivo studies in hypercholesterolemic rabbits at 6 months were performed.

Methods

All procedures were approved by the local ethics committee.

Animals

Twenty male New Zealand White rabbits (weighing 4.0±0.1 kg; Rykstasjon Kleinveeteelt, Merelbeka, Belgium) were used in this study. Ten rabbits were randomly assigned to a normal diet, whereas 10 others were fed a cholesterol-rich diet (0.3% cholesterol) for 6 months.\textsuperscript{8,9} At 6 months, all rabbits were prepared for intravascular ultrasound (IVUS) and thermographic assessment of the aorta. Briefly, the marginal ear vein was cannulated and the rabbit was anesthetized with sodium pentobarbital (30 mg/kg IV). After shaving the groin, the femoral artery was dissected, and a 6F sheath was introduced. Under fluoroscopy, a 0.014-inch guidewire (Guidant) was positioned in the right carotid artery; this was followed by both IVUS and thermographic measurements. Thereafter, the 10 normocholesterolemic rabbits and 5 randomly chosen hypercholesterolemic rabbits were put on a normal diet for another 3 months. At that time, all rabbits were again prepared for IVUS and thermography, followed by euthanasia for histological analysis.

Serum Samples

A serum sample was taken before and after lipid lowering. To address the relation between local temperature and local plaque composition, we performed ex vivo temperature measurements. An additional 3 male New Zealand White rabbits were fed a cholesterol-rich diet for 6 months; they were then euthanized after receiving a bolus of intravenous heparin (100 U/kg) to avoid immediate postmortem clot formation. The aorta of each animal was rapidly removed, and the proximal segment of the descending aorta was fixed to a corkboard with its dorsal side down. The thermography catheter was inserted, and the location of the 4 thermistors was marked on the vessel before pullback (described below), which was performed over a well-defined 3-cm segment from proximal to distal within 10 minutes after euthanasia.

IVUS

Pullback was performed using a 20-MHz, 3.2F IVUS catheter (CVIS, Boston Scientific Corp) with a predefined speed of 0.5 mm/s; it began at the ostium of the right carotid artery and lasted until the ninth costovertebral junction, which corresponded to the proximal part of the abdominal aorta (Figure 1). Images were recorded on videotape. IVUS characteristics of all aortic segments were carefully reviewed.

Thermography

Subsequently, a thermography catheter (Thermocore Medical Systems NV; Figure 2) was introduced and positioned at the same level as the IVUS catheter using fluoroscopy. The thermography catheter is an over-the-wire system that consists of a functional end that can be engaged by retracting a covering sheath. The distal part has 4 dedicated thermistors at the distal end of 4 flexible nitinol strips (each at 90-degrees) that, after engagement, with an expansion width of 9 mm, ensure endoluminal surface contact of the aorta. The thermistors are made of 5 kΩ bare chips (5 kΩm resistance; curve 7 material), with gold metallization and 40-American wire gauge wires soldered onto the metal; they can perform up to 25 measurements per second and are delivered with a certified accuracy of 0.05°C. After insertion into the abdominal aorta, the catheter was normalized to a randomly chosen thermistor in the abdominal aorta, it was positioned in the carotid artery, and its proximal part was then locked onto a dedicated pullback system (Thermocore Medical Systems). Pullback at a predefined speed (0.3 mm/s) was then initiated and stopped at the level of the ninth costovertebral junction (Figure 1). Pullback distances were divided into segments of 3 mm: with a pullback speed of 0.3 mm/s, and 25 measurements per second, each segment consisted of 250 measurements.

Ex Vivo Experiments

Once the thermistors were in place but before pullback, the immobilized aorta was marked at the start and longitudinally with different colors of indelible ink over 2 quadrants (north and east) over the complete length. After pullback, aortas were embedded and prepared for histopathological analysis. The first histological section corresponded with a section 0.5 cm distal from the start of the temperature measurements; sections were repeated every 0.5 cm. All histological sections were divided into 4 segments (quartiles), and plaque thickness was measured in all segments of all sections over the complete length of the analyzed aorta so that each histological segment precisely corresponded to the measured temperature with the thermistor at that particular point. Plaques were divided into 4 types according to plaque thickness and macrophage content, as described below.

The local temperature measurements from the 4 different thermistors were plotted against the pullback distance (3 cm); temperature measurements (derived at each histological segment) were related to the corresponding histology (plaque thickness and macrophage content) over the 4 different quadrants.

Histology

The following primary monoclonal antibodies were used: α-smooth muscle actin (Sigma) and M0/RAM-11 (anti-rabbit macrophages; Figures 3 and 5).

Figure 1. Anatomy of the rabbit aorta. The arrows indicate where registration of both IVUS and intravascular thermography started and stopped.

Figure 2. Close-up photograph of the thermography catheter illustrating the engaged distal end of the catheter, with the 4 thermistors widely expanded (each at 90°C). Scale bar is 1 cm.
Dako Glostrup). All antibodies were diluted in PBS. The monoclonal antibodies were detected by an indirect peroxidase antibody conjugate technique. The sections were incubated with a goat anti-mouse peroxidase antibody (Jackson Laboratories) for 45 minutes. To demonstrate the complex, 3-amin-9-ethylcarbazole was used as a chromogen. The specificity of the immunohistochemical reactions was checked by omitting the primary antibody and substituting an unrelated antibody for it at the same concentration.

Plaque thickness and the thickness of the superficial macrophage-rich layer were measured perpendicular to the endothelial surface in 12 randomly chosen sites per transverse section.9

In the ex vivo experiments, plaques were analyzed according to their thickness and their macrophage content along the complete analyzed segment. The cut-off value between thin and thick plaques was set at the median thickness of all plaques of the 3 cholesterol-fed rabbits and amounted to 140 μm. The cut-off value between plaques with low macrophage content and plaques with high macrophage content was set at the median number of RAM-11-positive cells per high-power field (40×; field diameter of 0.55 mm) of all plaques, which amounted to 15.

Statistics
Data are given as mean±SEM. To test whether the mean of the temperature differences in the aorta differed from zero, the one-sample t test was used. In vivo temperature differences in function of plaque thickness as well as ex vivo temperature measurements in function of plaque composition were evaluated with ANOVA followed by the Bonferroni test. Temperature differences and serum LDL cholesterol before and after lipid lowering were compared using the paired t test. The plaque thickness and the thickness of the superficial macrophage-rich layer before and after cholesterol lowering were analyzed using an unpaired t test. The SPSS 10.0 software package was used for all analyses. P<0.05 was considered significant.

Results
Procedural Outcome
All procedures were completed successfully, ie, at no point during or after the procedure did any adverse event occur in any of the 20 rabbits. Adverse events (death, stroke, infection, allergic reaction, or misbehavior) were not present.

Serum Lipids
LDL cholesterol levels after 6 months of a cholesterol-rich diet were 886±119 mg/dL; they were 91±78 mg/dL after 3 months of cholesterol lowering (n=5, P=0.002).

Histology
At 6 months, the aortas of control rabbits showed a normal vascular wall without plaque formation. Atherosclerotic plaques in the thoracic aorta of hypercholesterolemic rabbits were composed of both fibromuscular tissue and foam cells. Macrophages were found throughout the whole plaque or as a distinctive subendothelial layer (Figure 3A). After 3 months of cholesterol lowering, rare macrophages remained in the plaque (Figure 3B), although residual fat deposits could still be detected. The thickness of the plaques did not change after cholesterol lowering (Figure 3C). Macrophages, as demonstrated by a RAM-11 stain, were significantly reduced after cholesterol lowering (Figure 3D). Most of the remaining cells were spindle-shaped smooth muscle cells expressing α-smooth muscle actin (not shown).

IVUS
Pullback distance from the beginning of the carotid artery was 82±5 mm. Careful review of IVUS images taken in the normal rabbits revealed no plaques. In contrast, plaques could be recognized on IVUS in the carotid artery, aortic arch, and proximal part of the descending aorta in all atherosclerotic rabbits (Figure 4). Plaque formation was more pronounced in the aortic arch and proximal descending thoracic aorta compared with the distal thoracic aorta, thus confirming the histological data. On the basis of the thickness and resolution capacities of IVUS, plaques were divided into the following 3 groups: <200 μm, 200 to 400 μm, and >400 μm.

Thermography
Insertion of the thermography catheter into the carotid artery was feasible and safe in all rabbits. An example of a temperature measurement is shown in Figure 4. Average temperature on normalization of the thermistors (ie, the baseline core temperature in abdominal aorta) was 38.30±0.48°C for the nonatherosclerotic rabbits and 38.54±0.35°C for the atherosclerotic rabbits (P=0.691). Temperature heterogeneity was not present in the aortic segments of normal rabbits (mean temperature difference, 0.03±0.04°C; not different from zero; P=0.483). In hypercholesterolemic rabbits, mean temperature differences in the aorta increased with plaque thickness (Figures 4 and 5). However, after 3 months of dietary cholesterol lowering, temperature heterogeneity was significantly decreased, although plaque thickness remained unchanged (Figures 4 and 5).

Ex Vivo Experiments
Ex vivo experiments were performed at room temperature. The relation between local temperature and local plaque composition (plaque thickness and macrophage content) is shown in Figure 6, and an example is shown in Figure 7. The temperature of thick, macrophage-rich plaques differed significantly from thick, macrophage-poor plaques and from thin plaques.

Discussion
This study demonstrates for the first time that in vivo temperature heterogeneity is directly linked to atherosclerotic plaque composition. The temperature heterogeneity in this rabbit model of human-like atherosclerosis amounted to >1°C at sites of severe atherosclerotic plaque formation. This heterogeneity disappeared after decreasing the macrophage content of the plaques without influencing the thickness of the plaques by dietary cholesterol lowering. In addition, the use of the catheter system for detecting temperature differences in this animal model seemed to be safe and feasible.

Earlier histopathological studies have shown that human atherosclerotic plaques that rupture share certain common characteristics.4 These so-called vulnerable plaques typically consist of a thin fibrous cap covering a lipid-rich core. The cap is made of smooth muscle cells, extracellular matrix, and collagen fibers. The soft necrotic core underneath the cap contains an abundance of lipid-laden macrophages (derived from circulating monocytes) and a paucity of smooth muscle cells. A disturbance in the balance of the plaque composition...
induced either by proliferation of inflammatory cells or by the apoptosis of smooth muscle cells may be detrimental and lead to plaque rupture. The composition of atherosclerotic plaques of hypercholesterolemic rabbits at the level of the descending thoracic aorta shows some similarities with unstable human plaques. Indeed, these lesions are also composed of a collagen-rich matrix, a paucity of smooth muscle cells, and an abundance of macrophages. However, the necrotic core and fibrous cap are absent. Cholesterol withdrawal for 3 months resulted in drastic changes in plaque composition, with a significant decrease in macrophage content, without changing plaque thickness; this resembled the histological features of stable plaques.

Because previous ex vivo studies postulated that detecting the heat released by the activated inflammatory cells of atherosclerotic plaques may predict not only plaque rupture but also differentiate between stable and unstable angina, we analyzed in vivo the association between temperature heterogeneity and plaque composition in the aorta of hypercholesterolemic rabbits. The ex vivo experiments demonstrated that local temperature correlated with local plaque composition. Indeed, thick, macrophage-rich plaques showed a significantly higher temperature than any other plaque (including thick, macrophage-poor plaques), indicating that the total macrophage mass in a certain region determines temperature heterogeneity. The role of macrophages in explaining temperature heterogeneity was further underscored by the results of the dietary cholesterol lowering, which demonstrated that temperature heterogeneity decreased in parallel with the macrophage content of the plaques, although plaque thickness was unchanged.

In humans, the vulnerability of a culprit plaque is determined by a number of parameters, such as the critical mass of the lipid core, the thickness of the atheromatous fibrous cap, and the presence of an increased population of macrophages.
in the fibrous cap. The present animal study suggests that changes in the macrophage content in the plaque can be detected by in vivo thermography. This further indicates that the effect of cholesterol lowering on at least one parameter of plaque vulnerability can be evaluated in vivo. These data were obtained in an animal model that mimics some, but not all, features of human atherosclerotic lesions.

Our results demonstrate that the beneficial effect of cholesterol lowering would have been missed if the results were only based on measurements by IVUS or angiography. Indeed, acute coronary syndromes are often associated with angiographically nonsignificant coronary artery lesions and may simply result in a cardiac event based on a rupture of a mild atherosclerotic plaque. Mild atherosclerotic plaques may demonstrate temperature heterogeneity and, therefore, be considered unstable plaques. Therefore, rendering the plaque more stable and, thus, less prone to rupture by lipid lowering may be very helpful in reducing the amount of acute coronary syndromes.

Temperature heterogeneity may have important clinical implications because it may provide information about the plaque composition and, in part, its vulnerability, as opposed to more conventional diagnostic morphological and functional techniques. In this study, we showed that in vivo detection of temperature heterogeneity of atherosclerotic plaques is directly linked to plaque composition in a hypercholesterolemic rabbit model of atherosclerosis.

**Figure 4.** Examples of in vivo temperature measurements of the endoaortic surface in rabbits. A, In control rabbits at 6 months, temperature differences are absent along the aortic wall. IVUS image illustrates the absence of plaque formation. B, In atherosclerotic rabbits at 6 months, marked temperature variations up to \( \pm 1^\circ C \) are apparent along the endoluminal surface of the aortic wall. IVUS demonstrates plaque formation at the level of the proximal descending aorta just distal from the arch (arrow). C, In atherosclerotic rabbits after 3 months of dietary cholesterol-lowering, temperature heterogeneity is absent, although IVUS (taken at the same level as in B) demonstrates the presence of a similar plaque (arrowhead).

**Figure 5.** IVUS and temperature data from rabbit aorta with atherosclerotic plaques \((n=5)\). After 6 months of a cholesterol-rich diet (open bars), plaques of different thickness were present in the aorta. Temperature differences in the plaques increased with plaque thickness. After 3 months of dietary cholesterol lowering (solid bars), temperature heterogeneity had significantly decreased, although plaque thickness had not changed. Data are given as mean±SEM. **P<0.001 vs plaques <200 \( \mu m \); ++P<0.001 vs plaques of 200 to 400 \( \mu m \); P=0.01 vs before cholesterol lowering; and ##P<0.01 vs before cholesterol lowering.

**Figure 6.** Relation between local temperature measurement and local plaque composition (both thickness and macrophage content) derived from the ex vivo experiments \((n=3\) rabbits, 18 sections). Thick, macrophage-rich plaques had the highest temperatures of all plaques. \( P<0.001 \) vs no plaque and thin, macrophage-poor plaques; \( P=0.017 \) vs thin, macrophage-rich plaques; \( P=0.02 \) vs thick, macrophage-rich plaques.
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