Repeated Three-Dimensional Magnetic Resonance Imaging of Atherosclerosis Development in Innominate Arteries of Low-Density Lipoprotein Receptor–Knockout Mice

Paul D. Hockings, PhD; Toby Roberts, BSc Hons; Graham J. Galloway, PhD; David G. Reid, PhD; Dorothy A. Harris; Martin Vidgeon-Hart, AIBMS*; Pieter H.E. Groot, PhD; Keith E. Suckling, PhD; G. Martin Benson, PhD

Background—In vivo methods to evaluate the size and composition of atherosclerotic lesions in animal models of atherosclerosis would assist in the testing of antiatherosclerotic drugs. We have developed an MRI method of detecting atherosclerotic plaque in the major vessels at the base of the heart in low-density lipoprotein (LDL) receptor–knockout (LDLR−/−) mice on a high-fat diet.

Methods and Results—Three-dimensional fast spin-echo magnetic resonance images were acquired at 7T by use of cardiac and respiratory triggering, with 140-μm isotropic resolution, over 30 minutes. Comparison of normal and fat-suppressed images from female LDLR−/− mice 1 week before and 8 and 12 weeks after the transfer to a high-fat diet allowed visualization and quantification of plaque development in the innominate artery in vivo. Plaque mean cross-sectional area was significantly greater at week 12 in the LDLR−/− mice (0.14±0.086 mm² [mean±SD]) than in wild-type control mice on a normal diet (0.017±0.031 mm², P<0.01). In the LDLR−/− mice, but not control mice, increase in plaque burden at week 12 relative to week 1 was also highly significant (P=0.001). Lumen cross section was not significantly different between time points or groups. MRI and histological assessments of plaque size were closely correlated (R=0.8). The lumen of proximal coronary arteries could also be visualized.

Conclusions—This is the first report of in vivo detection of aortic arch atherosclerosis in any animal model. The method could significantly assist rapid evaluation of experimental antiatherosclerotic therapies. (Circulation. 2002;106:1716-1721.)

Key Words: magnetic resonance imaging ■ atherosclerosis ■ receptors ■ mice ■ arteries

There is a growing preclinical and clinical requirement for noninvasive imaging methods to characterize and measure atherosclerotic plaque in vivo. MRI may prove to be the method of choice. In the preclinical setting, serial measurements of atherosclerosis with the use of MRI could reduce the number of animals required for progression studies and lesion quantification time. Serial measurements would also facilitate disease regression studies. A key issue in atherosclerosis research is large interanimal variability in lesion size. If lesions could be measured in individual animals, each animal could act as its own control in longitudinal studies. MRI has the additional advantage that techniques and disease markers used in the laboratory are often directly relevant in the clinic.

Genetic mutations in the low-density lipoprotein (LDL) receptor gene cause severe hypercholesterolemia and atherosclerosis in humans, Watanabe heritable hyperlipidemic rabbits, and rhesus monkeys. LDL receptor–deficient (LDLR−/−) mice produced by homologous recombination develop modest hyperlipidemia and atherosclerosis on a normal diet, but serum cholesterol and atherosclerosis can be increased to a much greater extent than in normal mice by high-fat high-cholesterol feeding.

In LDLR−/− mice, as in other mouse strains, atherosclerotic lesions start to develop in the aortic root at the base of the heart. Indeed, current methods for measuring atherosclerosis in mice usually involve quantification of lesion cross-sectional areas in histological sections of the aortic sinus. However, recent reports suggest that the innominate artery, a short vessel connecting the aortic arch to the right subclavian and right carotid arteries, exhibits a highly consistent rate of lesion progression and that lesions in this region express many features that are relevant to the pathogenesis of clinically significant disease.
We used a 3D fast spin-echo (FSE) MRI method with cardiac and respiratory triggering to follow the progression of the atherosclerosis that develops in the innominate arteries of young LDLR−/− mice fed a high-fat high-cholesterol diet. The use of magnetic resonance (MR) to image vessels close to the heart is complicated by respiratory and cardiac motion. For this reason, previous studies have concentrated on vessels well removed from the thoracic cavity and have resorted either to examining very old animals or to inducing lesion development mechanically with a balloon catheter. As far as we are aware, this is the first MRI study of atherosclerotic plaque in the thoracic cavity in any animal model and the first to use 3D MRI with cardiac and respiratory triggering in free-breathing rodents.

Methods

All experiments complied with the UK Animal (Scientific Procedures) Act 1986 and the GlaxoSmithKline Code of Practice for Animal Experimentation.

Diet

The powdered RM1 diet is a standard rodent diet from Special Diet Services, and it contains 2.6% crude vegetable oil. The powdered semisynthetic high-fat/high-cholesterol/cholestrol (HFCC) diet was from Hope Farms and contained the following constituents (wt/wt): casein (20%), choline chloride 50% wt/wt (2%), methionine (0.2%), vitamin and mineral mixture (5.1%), sucrose (40.5%), corn starch (10%), corn oil (1%), cellulose (4.7%), cocoa butter (15%), cholesteryl (1%), and cholate (0.5%).

Mice

Pairs of homozygous LDLR−/− mice (Ldlr tm1Her), crossed over 3 generations onto a C57Bl/6J background, were purchased from Jackson Laboratories, Bar Harbor, Me, and bred by brother-sister mating and a minimal inbreeding system for 5 generations. Fifteen-week-old female wild-type (C57Bl/6J) (n = 9) and LDLR−/− (n = 9) mice were housed 3 per cage in a room that was lit from 6:00 AM to 6:00 PM and kept at 21 °C. All mice received food and water ad libitum and were fed the RM1 diet for 2 weeks before the LDLR−/− mice were weaned onto the HFCC diet.

MRI Protocol

Mice were imaged before the LDLR−/− mice were weaned onto the HFCC diet, and they were imaged again 8 and 12 weeks thereafter. Anesthesia was induced with 4% isoflurane in air/oxygen (1:1) and maintained with 1.5% isoflurane. The thorax was positioned over a 110-cm water pressure via a left ventricular cannula and outlet created by cutting the right atrium. After 30 minutes, the buffer was replaced with neutral-buffered formalin (10% formalin, Pioneer Research Chemicals) at 37 °C, and the perfusion was continued for a further 30 minutes. Bodies were then stored in the same formalin until they were processed.

Perfusion Fixation of Hearts and Aortas

After week-12 imaging, mice were euthanized by a rising concentration of CO2. Hearts and vascular were immediately perfused in situ with oxygenated Krebs-Ringer bicarbonate buffer at 37 °C under ~110-cm water pressure via a left ventricular cannula and outlet created by cutting the right atrium. After 30 minutes, the buffer was replaced with neutral-buffered formalin (10% formalin, Pioneer Research Chemicals) at 37 °C, and the perfusion was continued for a further 30 minutes. Bodies were then stored in the same formalin.

Dissection, Sectioning, Staining, and Analysis of Innominate Arteries

Heart, aorta, and all tissue from the base of the heart to the level of the thyroid gland were removed intact, including trachea, esophagus, carotid arteries, heart, thymus, and lungs. The lungs were discarded, and the heart was removed with ~1 mm of aorta attached at the base. The trachea, esophagus, carotid arteries, and thymus were then embedded in paraffin wax, and microtome trimming in the direction of the aortic arch produced transverse sections (5 μm) of the carotid and innominate arteries. Every 10th section was mounted onto glass microscope slides, dried overnight at 37 °C, stained with Coles hematoxylin and eosin, and imaged with the use of an Olympus SZH-10 stereo microscope with a ×0.5 objective (×20 overall magnification) and a video camera (Hitachi, HV-C10). The 24-bit color images were analyzed by using a PC (Datacell Pentium P5-133, Datacell) with a frame-grabbing board (Imaging Technology Inc Ic-PCI) and Optimas software (version 6.1, Optimas Corp.). Images were captured under identical lighting, microscope, camera, and PC conditions. Total innominate artery wall areas (media plus intima) were quantified by subtracting the lumen area from the total cross-sectional area of the vessel. Lumen area was calculated from the circumference of the lumen as though it was circular, as it is likely to be in vivo.

Results

Figure 1 compares multiplanar reformatted images from a typical wild-type mouse fed the RM1 diet for 12 weeks with images from a typical LDLR−/− mouse fed the HFCC diet for 0, 8, and 12 weeks (Figures 1A, 1B, 1C, and 1D, respectively). These paracoronal slices are in the plane of the aortic arch, with the branch points of the innominate and left carotid and left subclavian arteries clearly visible. After 12 weeks on the HFCC diet, plaque was clearly visible in the innominate arteries of the LDLR−/− mice. There was no change in the....

Hockings et al 3D MRI of Atherosclerosis in LDLR−/− Mice 1717
innominate arteries of the wild-type mice over the time course of this experiment (not shown).

Figure 2 shows multiplanar reformatted transverse sections from the same 2 mice at the level of the lines in Figure 1A and 1D; left and right images show wild-type and LDLR−/− sections, respectively. Histology and MR sections are taken halfway along the innominate artery at the midpoint between the junctions with the aortic arch and the right subclavian arteries. Details seen in the histological sections (top row) correlate well with details in the MR images (bottom). The panels in the second and third rows were reformatted from the 3D FSE images without and with fat suppression, respectively. The former give good delineation of the lumen, whereas suppression of perivascular fat in the latter allows better delineation of the adventitial surface of the vessel and, hence, the outer boundary of plaque plus vessel wall. The bottom row shows the combined non–fat-suppressed and fat-suppressed images, in which fat appears red. In the MR images, the arteries appear larger and more rounded than in the histological sections because they are under pressure and are dilated. Shrinkage in the formalin-fixed tissue before sectioning may also occur.

The length of the innominate artery ranged from 0.5 to 1.8 mm, so the results are reported as mean cross-sectional areas of plaque and lumen. Correlation between MRI and histological plaque measurements was good (Figure 3), with R = 0.8. Plaque areas measured by MRI were systematically lower than those measured by histology. This may be partially attributable to the difficulty of segmenting plaque adjacent to the regions of isointensity in the MR image, such as the thymus. Figure 4 shows that both MRI and histology detected highly significant plaque deposition in the LDLR−/− mice (MRI 0.14 ± 0.086 mm² [mean ± SD]; histology 0.308 ± 0.081 mm²) compared with the wild-type mice (MRI 0.017 ± 0.031 mm², P < 0.01; histology 0.075 ± 0.011 mm², P < 0.0001); MRI reveals an increase in plaque deposition in the LDLR−/− mice between week 1 and week...
12 \( (P=0.01) \), when there is no significant change in the wild-type mice \( (P=0.7) \). Lumen areas (wild-type areas \( 0.469 \pm 0.067 \text{ mm}^2 \), LDLR \( \text/\text/\text/ \) areas \( 0.426 \pm 0.061 \text{ mm}^2 \) ) are much higher by MRI (Figure 5) than histology, as expected from images acquired in systole compared with the flaccid postmortem condition. There was no significant difference between groups or time points in lumen area detected by either MRI or histology, suggesting some remodeling.

By use of the FSE technique, it was also possible to see, for the first time, the proximal sections of the coronary arteries in most mice (Figure 6). This figure demonstrates the high resolving power of the technique; however, this technique is not yet sufficient to differentiate the various plaque components in the mouse. There was some indication from the combined RGB images that the cap of the plaque seen in Figure 2 may have contained fat.

**Discussion**

In the present study, we obtained serial high-resolution images of atherosclerotic plaque as it developed in the innominate arteries of LDLR\(^{-/-} \) mice by using MRI methods that were completely noninvasive. Several groups have recently reported MRI visualization of atherosclerotic plaque in rodents and rabbits. Most describe imaging lesions formed after balloon angioplasty of the descending aorta in rabbits.\(^{13,14,18–20}\) However, Trouard et al\(^{21}\) could not visualize plaque in vivo in this model because of the low MR visibility of rabbit plaque lipids. MRI also failed to detect lesions induced by a silastic collar around rabbit carotid arteries because of insufficient resolution.\(^{22}\) Most studies in rats after balloon angioplasty\(^{23–25}\) report changes in lumen volume without direct plaque visualization. Using surgically implanted coils, only Arnder et al\(^{26}\) and Summers et al\(^{27}\) detected plaque in vivo in the rat. In neither of these 2 reports of lesion imaging in apoE-deficient mice was plaque development investigated. Fayad et al\(^{15}\) measured plaque in abdominal aortas and iliac arteries of old mice. Manka et al\(^{28}\) reported luminal narrowing in a restenosis model in the carotid artery but could not quantify plaque burden.

Our MRI methods were developed to optimize plaque visualization in the arteries at the base of the heart; effective suppression of signal from arterial blood is essential. The FSE sequence is useful in this respect because the signal from flowing blood is not refocused. We found maximal suppression of the signal from blood in the aortic arch and adjacent vessels with a 30-ms delay between the QRS wave and the beginning of the cardiac triggered FSE pulse sequence (43 ms to the center of the acquisition period), consistent with the study of Steinman and Rutt\(^{29}\) involving black blood MRI in humans, in which the optimal phase of the cardiac cycle to image the carotid arteries was found to be between peak systole and early diastole, when blood flow through these vessels is maximal. Imaging at this point has the further advantage that
vessels are fully dilated and relatively static during acquisition. Our FSE sequence acquired the signal for 26 ms of the 150-ms mouse cardiac cycle. Increasing the fraction of the cycle over which signal was acquired resulted in blurring and ghost artifacts.

When microscopic MRI was used to image aortas in rabbits and mice, the 2D slice selection methods resulted in highly anisotropic voxels (volumetric pixels with unequal dimensions),12,19,28 with maximum in-plane resolution of 360 μm (rabbits) and 48 μm (mice) and resolution in the third dimension of 3 mm and 0.5 mm, respectively. The use of relatively thick slices may result in so-called partial volume effects, in which the object of interest, eg, plaque, does not extend all the way through the imaging slice or is not perpendicular to the plane of the slice. Moreover, it can be difficult to reposition a 2D slice accurately. However, a 3D imaging lattice does not need to be repositioned with such accuracy because the isotropic block of MRI data acquired can be reformatted in any chosen orientation. For all these reasons, we chose a 3D approach in spite of its being more technically demanding.

During 3D acquisition, data are collected from all slices simultaneously, and motion between acquisitions results in ghosting and a loss of clarity of vessel walls. Visualization of plaque in the vessels at the base of the heart was possible only because data acquisition was synchronized with the cardiac and respiratory cycles to eliminate the effect of motion of the heart and thorax. With a typical heart rate of 400 bpm, accurate triggering proved critical to the success of the present study.20 Combined cardiac and respiratory triggering has rarely been used in atherosclerosis studies,26,27 and in those studies, rats were not breathing freely but were mechanically ventilated, an invasive intervention that complicates and slows down the preparation of animals for imaging. These studies used a 3D method to examine the rat carotid artery. By using an implanted coil, they were able to obtain resolutions of 25×25×400 and 32×32×500 μm, respectively. However, although resolution was superior to resolution in the present study (=140×140×140 μm), the implanted coil meant that the technique was invasive and had the potential to induce the formation of atherosclerotic lesions. Also, surgical intervention is often the rate-limiting step in the throughput of animals.

Previous MRI studies of experimental atherosclerosis have imaged lesions in the abdominal aorta or carotid arteries. However, either only very old animals could be used because lesion development in this area of the aorta is slow relative to the aortic root and adjacent arteries,12 or the rate of atherosclerotic lesion development had to be increased by removing the endothelial layer by use of a balloon catheter.14,19,26,28 In the present study, we have shown that diet-induced spontaneous lesion development can be studied if the appropriate imaging method is used to avoid the respiratory and cardiac motion.

In conclusion, it is possible to produce high-resolution (=140-μm) 3D images of atherosclerosis in vessels close to the heart in young LDLR−/− mice after 12 weeks on a high-fat diet. Combined cardiac and respiratory gating is crucial. The method is completely noninvasive in that there is no requirement for mechanical injury to arterial endothelium or use of implanted radiofrequency coils. Use of such MRI methods promises a more rapid assessment of efficacious plaque reduction therapies not only in the laboratory but also in the clinic.

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References
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