Noninvasive In Vivo High-Resolution Magnetic Resonance Imaging of Atherosclerotic Lesions in Genetically Engineered Mice

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Background—The pathogenesis of atherosclerosis is currently being investigated in genetically engineered small animals. Methods to follow the time course of the developing pathology and/or the responses to therapy in vivo are limited.

Methods and Results—To address this problem, we developed a noninvasive MR microscopy technique to study in vivo atherosclerotic lesions (without a priori knowledge of the lesion location or lesion type) in live apolipoprotein E–knockout (apoE-KO) mice. The spatial resolution was 0.0012 to 0.005 mm$^3$. The lumen and wall of the abdominal aorta and iliac arteries were identified on all images in apoE-KO (n=8) and wild-type (n=5) mice on chow diet. Images obtained with MR were compared with corresponding cross-sectional histopathology (n=58). MR accurately determined wall area in comparison to histopathology (slope=1.0, r=0.86). In addition, atherosclerotic lesions were characterized in terms of lesion shape and type. Lesion type was graded by MR according to morphological appearance/severity and by histopathology according to the AHA classification. There was excellent agreement between MR and histopathology in grading of lesion shape and type (slope=0.97, r=0.91 for lesion shape; slope=0.64, r=0.90 for lesion type).

Conclusions—The combination of high-resolution MR microscopy and genetically engineered animals is a powerful tool to investigate serially and noninvasively the progression and regression of atherosclerotic lesions in an intact animal model and should greatly enhance basic studies of atherosclerotic disease. (Circulation. 1998;98:1541-1547.)

Key Words: atherosclerosis ■ magnetic resonance imaging ■ genes

Genetically engineered animal models provide enormous potential for the study of the pathogenesis and treatment of numerous human diseases. The mouse is the most widely used animal for genetic studies, and techniques for genetic modification in vivo (transgenic and gene-targeting) are much more advanced in the mouse than any other mammal. For example, genetically engineered mice are being increasingly used as a model of atherosclerosis. Apolipoprotein E–knockout (apoE-KO) mice produced by gene-targeting technologies spontaneously develop atherosclerotic lesions similar in morphology to those observed in humans. Most experimental designs are typically limited to in vitro and ex vivo examination. However, it would be advantageous to develop an in vivo technique for serial, noninvasive imaging to monitor progression or regression of the arterial lesions in this and other mouse models. Such a technique would allow the performance of repeated analyses in the same animal, rather than study of multiple experimental groups with larger number of animals killed at different time points.

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that noninvasively identify and characterize atherosclerotic lesion burden without a priori knowledge of the lesion location or the lesion type.

Methods

Mice

The apoE-KO mice used in this study were generated by inactivating the apolipoprotein E gene by homologous recombination as described previously.3 The apoE-KO mice were from a colony of mice maintained at the Rockefeller University and characterized previously.13 A total of 8 apoE-KO mice (age, 36 to 84 weeks) were used. All wild-type control (n=5; age, 32 to 86 weeks) and apoE-KO mice were maintained on a regular chow (low-fat) diet. The mean plasma cholesterol level of apoE-KO mice was 600 mg/dL. Wild-type mice had a mean plasma cholesterol level of 100 mg/dL. The animal care was in accordance with institutional guidelines.

Before imaging, the mice were anesthetized with pentobarbital sodium (50 mg/kg IP; Nembutal, Abbott Laboratories). Pentobarbital provided ~1 to 2 hours of useful anesthesia. Two apoE-KO mice and 1 wild-type mouse died of anesthesia in the magnet after the end of the MRI experiment.

MRI

In vivo MR microscopy of the abdominal aorta and the common iliac arteries was performed with a Bruker 9.4-T, 89-mm-bore system operating at a proton frequency of 400 MHz (Bruker Instruments). A gradient insert (ID, 75 mm) capable of generating a maximum of 50 gauss/cm was used. The mice were placed in a 24-mm-ID tube and positioned head up in a vertical 25-mm-diameter RF imaging coil. The animals were maintained at a body temperature of 37°C throughout all imaging experiments with a thermocouple/heater system. Because of space limitations in the RF imaging coil, only mice with a body weight ≤29 g were imaged.

MRI parameters were selected to optimize visualization and characterization of the arterial wall. Each imaging session began with a multislice T1W spin-echo sequence to localize and select the area. All the images were magnified (×4) for alignment of the MR images with the corresponding histopathological sections.

Tissue Preparation and Histology

Surviving mice (10 of 13) were heparinized and then euthanized 20 minutes later with an overdose of ketamine. The chest was opened, and the aorta was cannulated via the left ventricle and perfused at 100 mm Hg with PBS (pH 7.4) containing heparin (100 U/L) until it was exsanguinated through an incision in the right atrium. Perfusion was then continued with 4% phosphate-buffered paraformaldehyde solution for 20 minutes. The posterior abdominal wall was removed en bloc, and fixation was continued for 24 hours. The blocks were then decalcified and transversely sectioned at intervals closely matching the corresponding MR cross sections. Each cross section was embedded in paraffin. Sections 5 μm thick were cut and stained with hematoxylin and eosin and a combined Masson's trichrome elastic stain for histopathological examination. Nonsurviving mice (3 of 13) were not perfusion-fixed but were processed for histology in a similar manner.

Image Analysis and Data Analysis

The raw data of the MR images were transferred to a computer and reconstructed with a custom program written with IDL (Research System Inc) running on a Macintosh computer. The histopathological cross sections were captured directly from a camera (Sony, 3CCD Video Camera) attached to a Zeiss Axioskop light microscope and transferred to a Macintosh computer.

The MR images were then correlated with the corresponding histopathological cross sections. We matched MR and histopathological cross-sectional images of the abdominal aorta and common iliac arteries from surviving apoE-KO (n=6) and wild-type (n=4) mice. Anatomic structures such as the aorta, common iliac arteries, abdominal aorta, and the common iliac arteries, spinal cord, back muscle, inferior vena cava, kidneys, renal pelvis, spleen, and the ureters were all used as external fiducial references for alignment of the MR images with the corresponding histopathological sections.

After agreement on MR and histopathological section alignment, 1 investigator (Z.A.F.) analyzed the MR data, and another investigator (J.T.F.) analyzed the histopathological data separately. Each was blinded to the results of the other modality.

By manual tracing of the lumen and the outer wall boundaries with ImagePro Plus (Media Cybernetics), the cross-sectional areas of the outer boundary and lumen of the vessel were measured on both the MR images and the histological sections. Wall area was calculated from the area of the outer wall boundary (total area) minus the lumen area. All the images were magnified (×15) during analysis, and the measurements were performed sequentially from the aorta to the common iliac arteries. The signal intensity of the sequential stack of MR images from the same mouse was scaled such that the visual contrast of soft tissues remained relatively constant.

The appearance of the atherosclerotic lesion shape in both MR and histopathological findings was evaluated and scored as 0, normal; 1, eccentric lesion; and 2, concentric lesion. The atherosclerotic lesion type was evaluated (1) for MR data according to its morphological appearance (lesion size and shape) and graded as 0, normal; 1, mild; 2, moderate; and 3, severe and (2) for pathological data and graded according to the American Heart Association (AHA) classification.14 The MR and histological findings (total area, wall area, lumen area, and lesion shape and type) were analyzed by simple linear regression with 95% CIs (Statview, Abacus Corp). Values are expressed as mean±SEM.

Results

Substantial respiratory motion artifacts in the MR images were detected above and close to the diaphragm. Minimal motion artifacts were noted below the level of the superior mesenteric artery; therefore, this region of the aorta down to the common iliac arteries was chosen for all subsequent MRI. As shown in Figure 1, the vascular lumen appeared dark in the MR images because of the blood flow–related signal loss from the spin-echo image acquisition. Structures such as the spinal cord, the inferior vena cava, the kidneys, the renal pelvis, and the ureters were clearly visible in all MR images (Figure 1). For example, the spinal cord appeared bright within the dark vertebra, which was surrounded by bright muscle. The kidneys exhibited a moderate gray level in the cortex. The corticomedullary boundary was distinguishable, as was the inner medulla of the renal pelvis. Although fat was suppressed, a thin layer of skin was resolved. The mouse's tail, folded forward onto the abdomen, is visible at the 9 o'clock position in Figure 1D.

The abdominal aorta and the common iliac arteries were visualized by MR in all 13 mice. In 1 of the apoE-KO mice, artifacts due to the improper tuning of the 90° and 180° RF imaging pulses partially obscured the atherosclerotic lesions in the images.
Fifty-eight MR/histopathology–matched cross sections of the aorta and common iliac arteries were analyzed. All wild-type mice \((n = 5)\) were free of atherosclerotic lesions, as shown on MR images in A and B (magnified, see scale) and histopathology (C), as shown by hematoxylin-eosin stain (magnification \(\times 40\)). Large atherosclerotic lesion (arrow) that encircles abdominal aorta of 12-month-old apoE-KO mouse is shown on MR images in D and E (magnified). These findings correlated with histopathology, as shown in F (hematoxylin-eosin stain; magnification \(\times 40\)). All MR images have pixel size of \(97 \times 97 \times 500 \mu m^3\). Left kidney and spinal cord are used as anatomic landmarks to facilitate comparison between MR images and histological sections.

**Figure 1.** MR images (PDW) of abdominal aorta (arrow) in normal mouse and apoE-KO mouse showing differences between normal and atherosclerotic arteries. On all MR images, lumen is dark. Normal abdominal aorta wall thickness is \(\approx 50 \mu m\) and was not clearly visible at spatial in-plane resolution of \(97 \mu m\). Wild-type mice were free of atherosclerotic lesions as shown on MR images in A and B (magnified, see scale) and histopathology (C), as shown by hematoxylin-eosin stain (magnification \(\times 40\)).

The wall area of the abdominal aorta in apoE-KO mice (Figures 1D and 1E) was significantly increased compared with the aortas of wild-type mice. In all apoE-KO mice, morphological measurements by MR \((n = 39)\) were consistently larger compared with histopathological analysis \((n = 45)\) (Figure 4). With MR, the total area was \(0.622 \pm 0.056 mm^2\), the wall area was \(0.384 \pm 0.046 mm^2\), and the luminal area was \(0.238 \pm 0.020 mm^2\). With histopathology, the total area was \(0.499 \pm 0.045 mm^2\), the wall area was \(0.300 \pm 0.035 mm^2\), and the luminal area was \(0.200 \pm 0.021 mm^2\). There was good correlation between MR measurements and histopathological analysis of wall area (Figure 4) and total area (slope = 1.0 for wall area, slope = 0.94 for total area, and correlation coefficient of 0.86 for both). Linear regression analysis between MR and histo-
logical measurements of luminal area showed that the correlation coefficient was 0.58 (slope=0.54).

There was excellent agreement between MR and histopathology in all mice (n=58 cross-sectional images) in the grading of lesion shape (slope=0.97, correlation coefficient=0.91, n=58). Eight atherosclerotic lesions identified by histopathology as eccentric were graded by MR as normal, and 1 atherosclerotic lesion histopathologically identified as eccentric was graded by MR as concentric (Table 1).

All 20 aortic and iliac artery sections from apoE-KO mice identified by histopathology as normal were also graded as normal by MR. Two AHA type I lesions (wall area, 0.01 and 0.03 mm$^2$) identified by histopathology were graded by MR as normal, and 3 AHA type II lesions (wall area, 0.15, 0.19, and 0.20 mm$^2$) were graded by MR as normal (Table 2). Linear regression analysis performed between MR and histopathology gradings of atherosclerotic severity showed a close positive correlation (slope=0.64, correlation coefficient=0.90, n=58).

Discussion

To the best of our knowledge, this is the first report of the feasibility of in vivo MR microscopy to visualize the arteries of wild-type and apoE-KO mice and to identify and characterize atherosclerotic lesions when present, without a priori knowledge of the lesion location or the lesion type. Our study demonstrates that atherosclerotic burden in terms of lesion location, size, and type can be accurately quantified by MRI of living apoE-KO mice. The lesion was characterized on MR images by use of a grading system to estimate the shape and severity of the atherosclerotic burden. There was a high level of agreement between MR and histopathological findings.

Our study revealed that normal aortic lumen area was maintained in apoE-KO mice as measured by both MR and histopathology (0.238±0.020 mm$^2$ in apoE-KO mice versus 0.241±0.037 mm$^2$ in wild-type mice). This was probably the result of remodeling, as recently reported in histopathological studies in apoE-KO mice.

The vessel wall was scored for apparent degree of severity in MR images and was later verified by AHA classification criteria in histopathological cross sections. This is relevant in the assessment of progression or possible regression of atherosclerosis in the same animal.

High-quality MR images of the abdominal aorta and common iliac arteries were obtained free of respiratory motion and artifacts without respiratory triggering. This was the result of the intraperitoneal injection of pentobarbital, which produced low-amplitude motion in the thorax and abdomen as well as the tight constriction afforded by the mouse holder. A longer-lasting anesthetic, such as isoflurane inhalation anesthesia, would allow longer imaging time. However, this anesthetic results in large-amplitude breathing motions in the mouse thorax and abdomen and requires complicated respiratory gating methods to obtain useful images.

An in-plane spatial resolution of 97 μm was adequate to visualize and quantify atherosclerotic lesion morphology in apoE-KO mice, as evidenced by the high correlation coefficients between the MR and histopathological measurements (Figure 4). The systematic overestimation of lesion size by MR compared with histopathological analysis is probably the result of specimen shrinkage that occurs during histological preparation and of volume-average effects (caused by the slice thickness and imaging plane orientation). At an in-plane spatial resolution of 97 μm, a mean wall area of 0.384±0.046 mm$^2$ was required to permit definitive delineation of the vessel wall. Higher-resolution imaging (48 μm) allowed the definitive delineation of the very-thin-walled aorta in the wild-type mice (wall area, 0.056±0.008 mm$^2$). The measured wall area in apoE-KO mice (36 to 84 weeks old) is in general agreement with published data.

Figure 2. MR image (PDW) of abdominal aorta (arrow) in same normal mouse and same location as in Figure 1A and 1B but with pixel size of 48×48×500 μm$^3$. Normally thin-walled abdominal aorta is clearly visible in A and B (magnified, see scale). Because of smaller pixel size, signal-to-noise ratio in this image is lower than in Figure 1.
accurately depicted eccentric lesions (Table 1), a subgroup of potentially unstable lesions in humans.\textsuperscript{21}

The different section thicknesses of the histopathology (5 \(\mu m\)) and the MR (500 \(\mu m\)) may have resulted in registration errors and in measurement errors. We carefully coregistered the MR images with the corresponding histological sections by using anatomic structures as external fiducial references. Thinner slice thickness of MR images, such as those possible with 3-dimensional acquisition techniques,\textsuperscript{12} may further improve coregistration and reduce partial-volume effects, but this was not a real problem in our study.

**Study Limitations**

Vessel motion during the cardiac cycle is a source of MR image artifacts (eg, vessel wall motion). ECG gating can be used as an approach to pulsatility problems and has been reported in mice.\textsuperscript{10} However, because of limited hardware and software capabilities, we did not study the effect of ECG

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**Figure 3.** T1-weighted MR images (magnified, see scale) of atherosclerotic lipid-rich complex lesions in apoE-KO mice. Pixel size of MR images is 97\(\times\)97\(\times\)500 \(\mu m^3\). At top left of abdominal aorta, an atherosclerotic small branch vessel (br) is seen by MR (A, white arrow) and by histopathology (B) in 21-month-old apoE-KO mouse. This lesion had a focal calcium deposit in abdominal aorta (yellow arrow) that appeared as a signal void (A) and correlated with histopathological findings (B), as shown by hematoxylin-eosin stain (magnification \(\times 40\)). Inferior vena cava (IVC) is shown at right of abdominal aorta (A). Atherosclerotic lesions in right (r) and left (l) common iliac arteries (white arrows) in a 17-month-old apoE-KO mouse are detected with MR (C) and correlated with histopathological findings (D, hematoxylin-eosin stain; magnification \(\times 15\)).
TABLE 1. Atherosclerotic Lesion Shape by Histopathology and MR From Cross-Sectional Images of the Abdominal Aorta and Common Iliac Arteries in Wild-Type and apoE-KO Mice

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Figure 4. Linear regression analysis showing good correlation between measurements of wall area by MR and histopathology from cross-sectional images of abdominal aorta and common iliac arteries in wild-type and apoE-KO mice. Dashed lines indicate 95% CIs.

gating on our MR images of the abdominal aorta and common iliac arteries.

Other sources of imaging artifact arise from unsaturated blood flow, which can obscure the detection of vessel wall lesions. This occurred in 1 of the apoE-KO mice because of the improper tuning of the 90° and 180° RF imaging pulses. However, the rest of the studies were free of blood-flow artifacts. Improved inflow saturation methods that eliminate the luminal blood-flow signal are being investigated.

Differentiation of lipid and fibrous components present in the atherosclerotic lesion was not possible because of the large pixel sizes used in this study and the limited signal-to-noise ratio, especially in the T2-weighted images necessary for imaging of these components, as demonstrated previously. However, calcified components, because of their low signal on both PDW and T1W images, were detected in some of the MR images (Figure 3).

Ongoing methodological improvements in signal-to-noise ratio, image resolution, and image acquisition speed will increase the level of detail obtained in future MRI studies. Additional MR techniques, such as water diffusion weighting, magnetization transfer weighting, and contrast enhancement, may also provide complementary structural information and allow detailed atherosclerotic plaque characterization. Nonetheless, our results of atherosclerotic lesion size, shape, and burden clearly demonstrate that the combination of MR microscopy and genetically engineered mice is a powerful tool for the serial, noninvasive testing in the intact animal of the effects of environment, hormones, drugs, and genes with the potential to alter disease. Such advances in imaging should greatly accelerate our understanding of the pathogenesis, diagnosis, and treatment of atherosclerosis in the near future.

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