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Use of Nuclear Magnetic Resonance Imaging Angiography to Follow-Up Arterial Remodeling in an Animal Model

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ABSTRACT

Appropriately sized arteries in small animals may be possible models for studying the remodeling process as occurs after arterial balloon injury in humans. Magnetic resonance imaging (MRI) is able to noninvasively image tissue in vivo. To date, small animal angiography models have mostly used research-dedicated instruments and resolution, which are not universally available.

Experiments were carried out on a rat aorta model of remodeling in vivo (n=40). Arteries were injured by oversized balloon dilation; control arteries were uninjured. Angiography imaging was performed immediately before sacrifice with an unmodified clinical MRI unit, a 1.5 Tesla MR tomograph with a 20-cm-diameter coil. Longitudinal MRI pictures of the aorta and morphometry of tissue sections to measure luminal and arterial wall areas were analyzed with use of computer-assisted techniques.

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(Abstract continued)

Comparison of dimensions demonstrated correlation between MRI and histology measurements of the lumen. MRI and morphometry showed a gradual increase in mean luminal area over 6 weeks following injury. The lumen increase correlated with total arterial area and thickness.

In this rat aorta model, remodeling documented at histology was followed-up in vivo. The use of such clinical MRI scanners has potential to reduce animal numbers needed to follow-up the remodeling process after therapeutic intervention.

Introduction

Arterial renarrowing after angioplasty has previously been attributed solely to intimal thickening, consisting of cell proliferation and matrix formation.[1] Animal models for investigating potential therapies for restenosis have concentrated, therefore, on mimicking this component.[2] The rat carotid model has been commonly used for investigating antiproliferative therapies, for many animals can be processed in a short time to give early results of effectiveness. It has become clear that increased wall thickness is not the only determinant of late lumen dimensions and restenosis in patients and that geometric remodeling plays a role.[3-5] Vascular remodeling is an active process of structural alteration of vessels in response to hemodynamic conditions and local injury.[6] Up until now, no optimal experimental model has been described in which the remodeling can be followed-up in vivo, although several angiographic and histologic studies have documented that it also occurs in rabbit and porcine arteries after injury.[7-10] Potential therapies targeting remodeling after angioplasty should be tested in a suitable model.

Contrast angiography is the standard method of assessing lumen diameter in vivo in humans. Intravascular ultrasound gives additional information about wall and lumen dimensions.[4] Both angiography and intravascular ultrasound require arterial access and are limited for follow-up studies. For repeated investigations evaluating the development of remodeling, a noninvasive method would be preferable. The patency of vessels as small as human coronary arteries has been studied by magnetic resonance imaging (MRI), with a high sensitivity and specificity compared with standard invasive coronary angiography.[11] MRI of explanted human atherosclerotic arteries correlates with measurements obtained at histology.[12] Magnetic resonance microscopy for follow-up of rat carotid artery injury has been carried out through use of implanted imaging coils.[10]

In this article, we describe the use of a clinical MRI scanner to follow the remodeling process in a small animal model. A good correlation with morphometric results in a large number of animals would allow repetitive noninvasive investigations of small vessels at various time points, leading to a reduction of animals needed for vascular experiments.

Materials and Methods

Experimental Protocol

Adult Sprague-Dawley rats with 500 g body weight (n=30) fed a normal chow diet were anesthetized with intraperitoneal pentobarbital 40–60 mg/kg. With use of standard sterile technique, the femoral artery on one side was exposed. A 3 mm Grüntzig balloon angioplasty catheter was introduced via a femoral artery arteriotomy into the abdominal aorta to a set distance and inflated for 1 minute. The balloon was deflated and removed. The femoral artery was tied off, the wound closed, and the animals were allowed to recover. Uninjured aortae from normal rats were used as controls (n=10).

Magnetic Resonance Imaging Technique

Normal or experimental rats were anesthetized as before and imaged with a clinical MRI scanner at 2 weeks (n=20), 4 weeks (n=6), or 6 weeks (n=4) following injury. Normal rat aortae (n=10) were similarly scanned. The MRI was performed on a general-purpose scanner with a magnetic field strength of 1.5 Tesla (ACS2, Philips Medical Systems, Best, Netherlands) with

a 20-cm-diameter extremity coil. High-resolution images were acquired with a dedicated coil normally used for human knee imaging (diameter 20 cm) by means of a time-of-flight imaging sequence where flowing blood is enhanced in contrast to normal tissue. Minimal respiratory motion artifacts were recorded and the fast heart rate did not cause variability in measurements. Standard anatomic features, including the diaphragm, renal arteries, and the aortic bifurcation, were delineated at the start of the examination and used to orientate the rest of the pictures. Twenty-five slices with a matrix size of 512×512 and a field of view of 120 mm, resulting in an in-plane resolution of 234 microns, were imaged. Axial slices were 2 mm thick and separated by a 1.3 mm gap; this enabled imaging from 15 mm below the aortic arch down to 20 mm below the bifurcation, ensuring inclusion of the entire abdominal aorta. The 20 mm repetition and echo times were 45 ms and 12 ms, respectively; a flip angle of 60° was used. Four measurements were performed and averaged. Total acquisition time was approximately 12 minutes per animal.

Angiographic maximum intensity projections were generated on the MR tomograph console. The internal diameter of the vessel was acquired by measuring where blood flow could be visualized in both coronal and sagittal planes with use of computer-assisted analysis, thus deriving an estimate of aortic lumen area at defined levels (Figure 1). Measurements were made by two independent reviewers, blinded to the animal group, and the mean of the results was used for the statistical analysis.

Histology

Animals were immediately sacrificed after imaging and arteries were perfusion-fixed with use of 4% paraformaldehyde/phosphate-buffered saline, processed, and frozen at –80°C for histology. The elasticity of the aorta appeared preserved, despite injury, and there appeared to be no distortion of the normal anatomy. Careful measurement and marking of the aortic segments ensured that each could be matched to the MRI pictures later.

Arterial wall thickness and lumen dimensions were measured at histology. Cryostat sections of 10 μm were stained with elastic trichrome and microscopic pictures transferred with a photographic system (Nikon Type 104, Düsseldorf, Germany) onto a computer. The same computer-assisted analysis program as used for the MRI images was used to measure the aortic lumen in pixels, which could be converted into mm^2 after measurement of a standard.

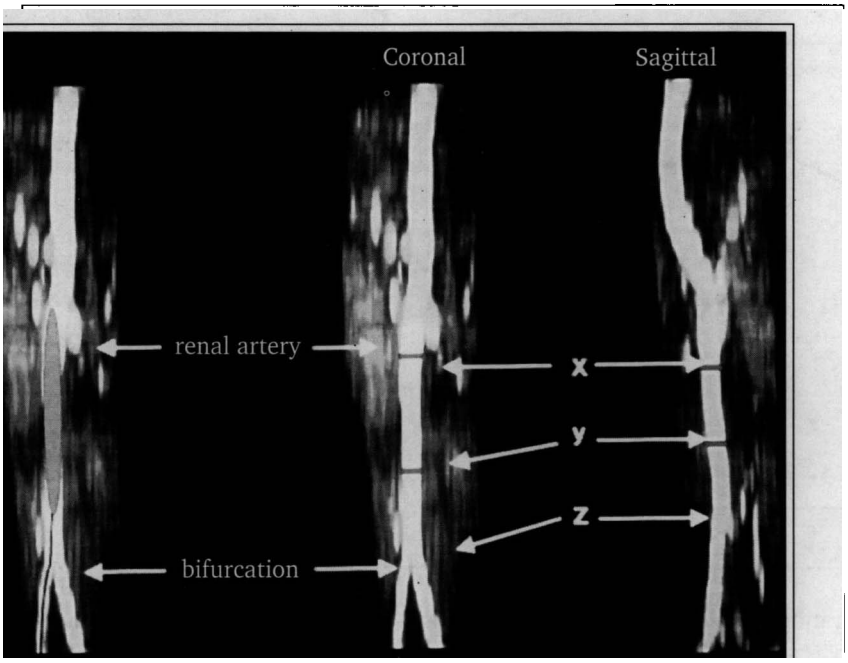


Figure 1. Longitudinal view of aorta at MRI angiography demonstrating the injury model (left) and levels at which measurements of lumen were made in each animal (points x, y, and z) demonstrated in sagittal and coronal views. Point x is immediately below the lower renal artery; point z was immediately above the bifurcation; and point y was in the middle of these two markers. The mean of the values obtained at points x and y was used to represent an approximation of the value at the site of maximal injury.

Data Analysis

Normal and injured rat aortae were used to compare MRI pictures of the aorta with histologic measurements of arterial dimensions. Lumen dimensions by MRI were calculated from longitudinal views, diameter measurements being taken in two planes (Figure 1). The correlation between MRI and histology was performed by use of reciprocal linear regression analysis. Arterial wall thickness and lumen dimensions at histology were also compared by use of regression analysis. Comparisons of dimensions over time periods analyzed were made with a one-way analysis of variance with Scheffes F-test. Results were expressed as mean ±SEM for each group. Statistical significance was set at $p<0.05$.

Results

There was no difference in weight at sacrifice between rats of different groups (weight 560 ±54 g in the injury group and 548 ±34 g in the normal uninjured group, p=ns). MRI acquisition was relatively simple after a standard anesthetic protocol. The size of the coil enabled two rats to be imaged at the same time. Imaging was within 20 minutes in all cases.

Comparison of lumen dimensions demonstrated correlation between MRI and histology measurements ($r=0.60$, $p<0.001$) (Figure 2). With increasing time after balloon injury, MRI showed a gradual increase in mean luminal area over 6 weeks following injury (2.75 ±0.20 mm^2 normal vs 4.71 ±0.59 mm^2 6 weeks post-PTA; $p<0.05$), confirmed by use of morphometry (0.82 ±0.06 mm^2 vs 1.34 ±0.08 mm^2; $p<0.05$) (Figure 3). The area within the external elastic lamina was larger and the arterial wall was thickened in comparison with normal arterial measurements at histology: area within the external elastic lamina (EEL) (1.00 ±0.66 mm^2 vs 1.94 ±0.17 mm^2; $p<0.05$) and wall thickness (0.17 ±0.02 mm^2 vs 0.60 ±0.09 mm^2; $p<0.05$). The lumen increase correlated well with total arterial area and thickness ($r=0.83$; $p<0.001$) (Figure 4).

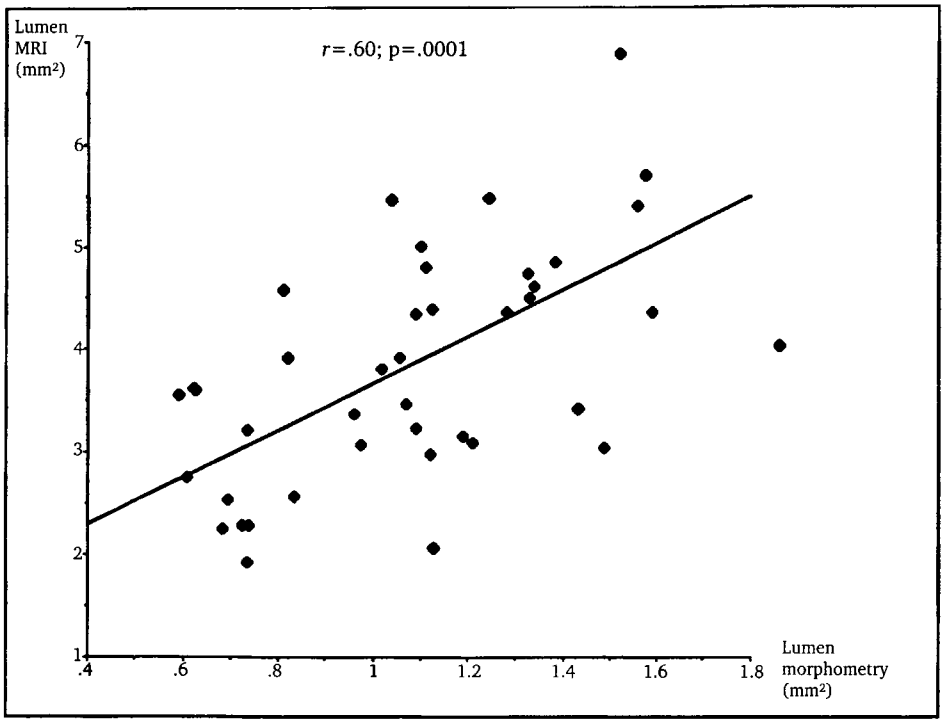
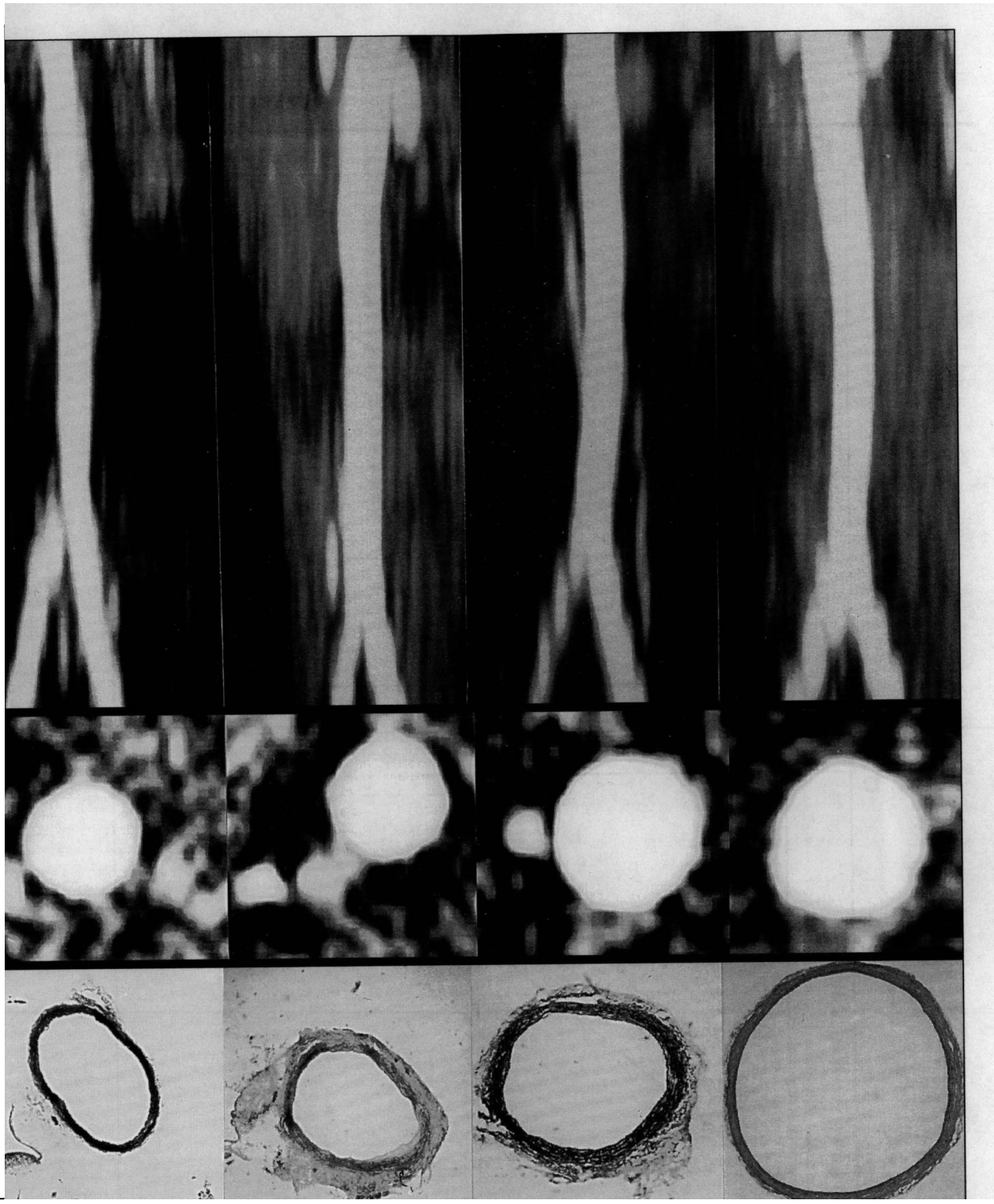
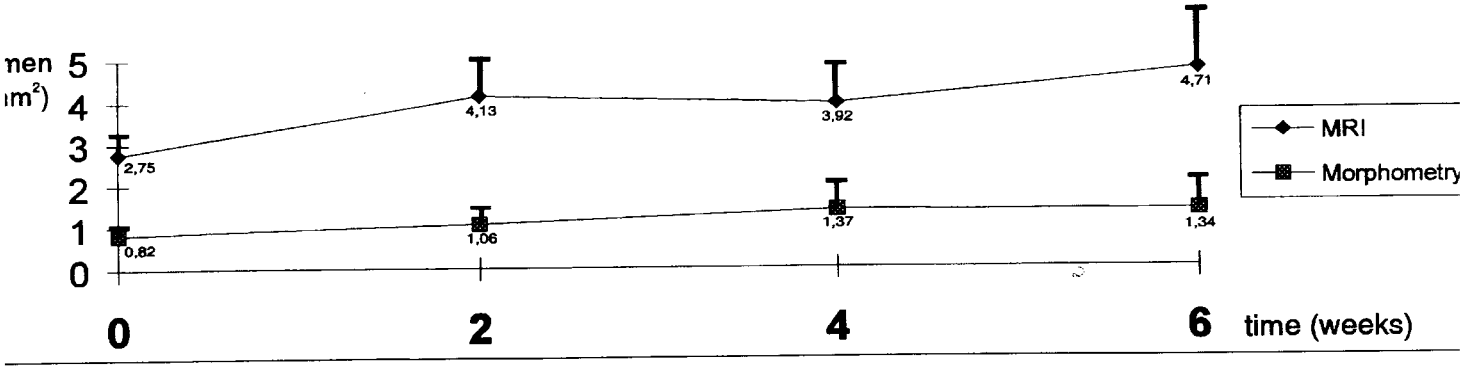


Figure 2. Regression analysis demonstrating correlation between lumen areas measured by use of MRI and morphometry ($r=0.60$; slope $Y=1.40 + 2.27 \times X$; $p<0.001$).

Figure 3.
(opposite page):
Lumen measurements by MRI and histology in normal rats and 2, 4, and 6 weeks after injury. (a) Longitudinal pictures of magnetic resonance angiography (1 mm=0.28 mm in reality); (b) axial slices at defined point X (1 mm=0.08 mm in reality); (c) histology at the same level (magnification 36×); (d) graph demonstrating a gradual increase in mean luminal area over 6 weeks following injury at MRI (2.75 ±0.20 mm^2 normal vs 4.71 ±0.59 mm^2 SEM 6 weeks post-PTA; $p<0.05$), confirmed by morphometry (0.82 ±0.06 mm^2 vs 1.34 ±0.08 mm^2 SEM; $p<0.05$).





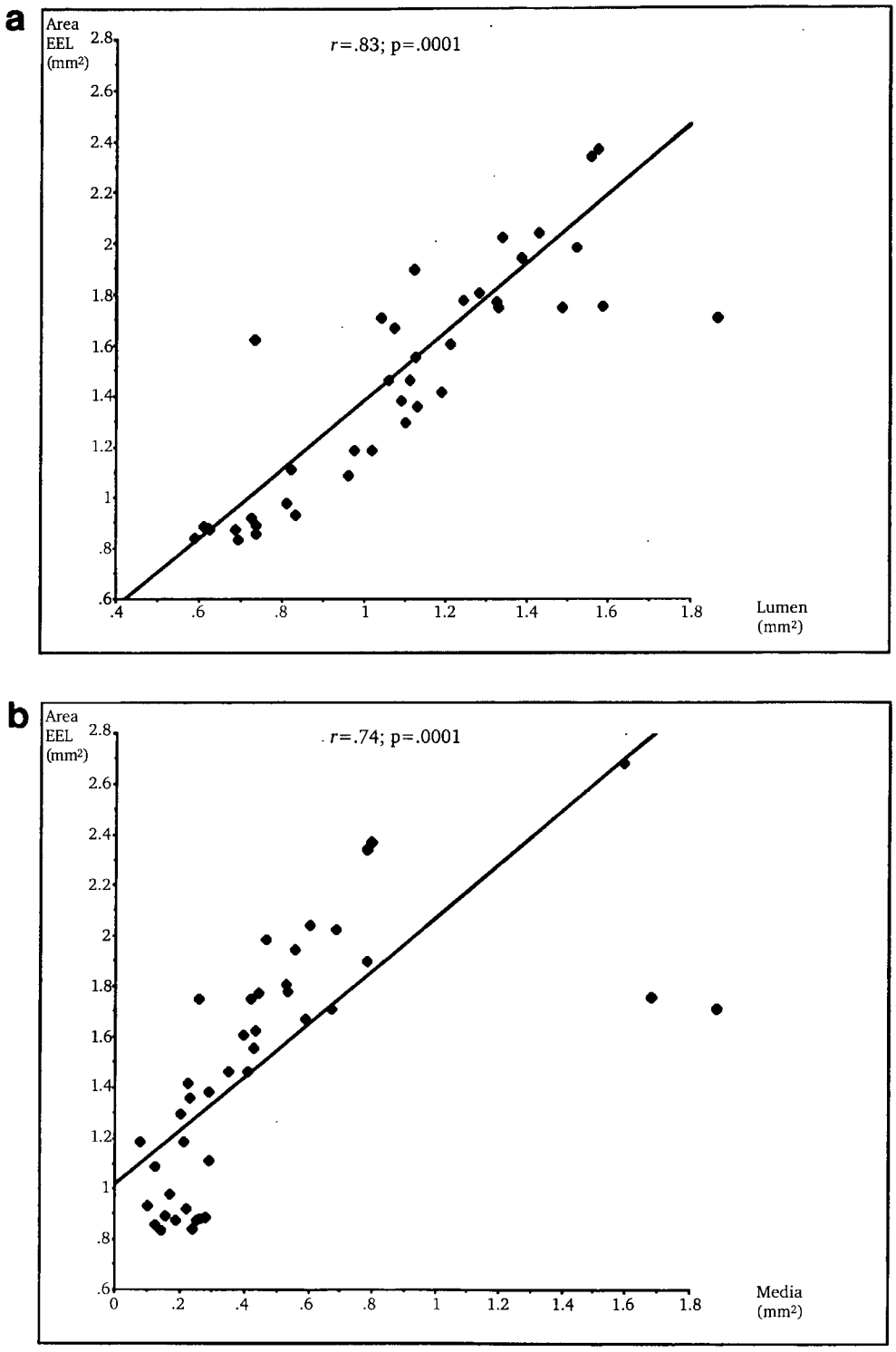


Figure 4. Regression analyses demonstrating correlation between lumen area and area within the external elastic lamina (a) (r=0.83; slope Y=0.03 + 1.35 × X; p<0.001) and medial area and area within the external elastic lamina (b) (r=0.74; slope Y=1.02 + 1.05 × X; p<0.001) measured at morphometry. Overall, in injured vs normal aortae, there was a significant increase in t area within the external elastic lamina (EEL) (1.00 ±0.66 mm² vs 1.94 ±0.17 mm² SEM; p<0.05) and the wall thickness (0.17 ±C mm² vs 0.60 ±0.09 mm² SEM; p<0.05).

Discussion

Use of MRI to noninvasively image arteries both ex vivo and in vivo in experimental animals has concentrated predominantly on wall structure and components, especially the detection and characterization of atherosclerotic plaque. The chemical content of atherosclerotic plaque in isolated human arteries obtained at autopsy can be analyzed by MRI spectroscopy in vitro.[13] This methodology can discriminate between individual arterial wall layers, as well as identify lipid core and collagenous cap within plaque.[14] Results in excised arteries might be applicable to in vivo studies in the future, although the need for adaptation of clinically available scanners must first be addressed. Skinner et al used an in vivo rabbit model of atherosclerosis to follow the development of atheroma.[15] In this longitudinal study, potentially dangerous features of plaque development were successfully imaged. In arteries of this size, lumen dimensions acquired at MRI appeared to correlate with histologic measurements. MRI has previously been used to visualize normal and aneurysmal rat aorta in vivo.[16] This was correlated with visual assessment of the outer aortic diameter measured with calipers under direct vision after laparotomy, carried out within a week of the scanning. In our study we sacrificed the rats immediately after the scan and used standard MRI scanning techniques. MR angiographic time-of-flight was chosen for the imaging sequence, for traditional, morphologic sequences are not able to resolve the arterial walls in the absence of plaques in a whole-body scanner. In this case the fatty components can be enhanced and visualized. Using time-of-flight procedures instead allows for delineation of the active vessel lumen through enhancement of flowing blood.

Remodeling was first described in association with atherosclerosis, where progressive intimal thickening over time leads to adaptive changes in arterial size, such that the lumen is relatively preserved despite large plaque loads.[17] Changes in cellular and noncellular components interact to alter lumen and wall dimensions over time. Fundamental differences clearly exist between species after balloon injury. In the rat aortic model, we have shown by histology that the arterial wall is thicker after injury through an increase in cell numbers in-between elastic fibers, a difference that is increased after a longer time. At the same time, the morphometric measurements indicate that the entire artery and lumen areas increase in size to accommodate this wall thickening, resulting in aneurysmal remodeling. A variable "aneurysmal" dilatation has also been documented as a response to injury in a certain proportion of atherosclerotic rabbit aortae[15] and femoral arteries[9] after injury and seems to be more typical in elastic arteries in contrast to muscular arteries such as coronary arteries. In other animal models, severe reaction to injury also involves some change in overall vessel size, with constriction of arteries. This is especially true of muscular arteries like the coronary vessels.[8] A primate model using long-term high-cholesterol feeding appears to closely resemble human remodeling;[18] however, such large animal models are not cost-effective for preliminary studies.

If remodeling is an important component of restenosis, there is now potential to evaluate and target relevant proteins as part of this process. Influencing remodeling could ensure adaptive change is beneficial and that lumen dimensions are preserved. This study demonstrates that a conventional clinical MRI scanner is sensitive enough to quantify changes in lumen area in the rat aorta in vivo, confirming results obtained by histology of tissue sections. Moreover, lumen diameters measured are consistent with those found in normal and aneurysmal rat aorta in vivo (1–3 mm).[16] In this previous study, MRI measurements were correlated with direct measurements made visually at laparatomy and differences averaged 0.16 mm with $r=0.98$ and $p<0.001$. In our animals, differences in measurements were greater because of the elasticity of arteries, resulting in shrinkage following explantation and fixation compared with in vivo conditions where arteries are perfused with normal blood pressure; despite this, good correlation was found.

The MRI method of evaluating lumen size could thus be used to follow-up remodeling after injury. Repeated measurements can be made noninvasively at different time points in the same animals even in small animals such as rats. The model was practical: large numbers of animals were processed to validate the imaging method, with a comparatively short imaging time. The rat aorta model resulted mainly in expansion of the lumen after angioplasty and to a lesser extent in an increase in wall thickness: a possible model for the remodeling process. In contrast to observations made with MRI on atherosclerotic arteries,[15] it was not possible to visualize the thin arterial

wall in such detail in this rat aorta model. However, a simple model like this is ideal for carrying out preliminary studies and following-up lumen dimensions over time up until sacrifice. The imaging method will ultimately enable sparing of animal numbers and cost reduction.

Acknowledgments

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