Molecular Imaging

In Vivo Assessment of Aortic Aneurysm Wall Integrity Using Elastin-Specific Molecular Magnetic Resonance Imaging

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Background—The incidence of abdominal aortic aneurysms (AAAs) has increased during the last decades. However, there is still controversy about the management of medium-sized AAAs. Therefore, novel biomarkers, besides aneurysmal diameter, are needed to assess aortic wall integrity and risk of rupture. Elastin is the key protein for maintaining aortic wall tensile strength and stability. The progressive breakdown of structural proteins, in particular, medial elastin, is responsible for the inability of the aortic wall to withstand intraluminal hemodynamic forces. Here, we evaluate the usefulness of elastin-specific molecular MRI for the in vivo characterization of AAAs.

Methods and Results—To induce AAAs, ApoE-/- mice were infused with angiotensin-II. An elastin-specific magnetic resonance molecular imaging agent (ESMA) was administered after 1, 2, 3, and 4 weeks of angiotensin-II infusion to assess elastin composition of the aorta (n=8 per group). The high signal provided by ESMA allowed for imaging with high spatial resolution, resulting in an accurate assessment of ruptured elastic laminae and the compensatory expression of elastic fibers. In vivo contrast-to-noise ratios and R1-relaxation rates after ESMA administration were in good agreement with ex vivo histomorphometry (Elastica van Gieson stain) and gadolinium concentrations determined by inductively coupled plasma mass spectroscopy. Electron microscopy confirmed colocalization of ESMA with elastic fibers.

Conclusions—Changes in elastin content could be readily delineated and quantified at different stages of AAAs by elastin-specific molecular magnetic resonance imaging. ESMA-MRI offers potential for the noninvasive detection of the aortic rupture site prior to dilation of the aorta and the subsequent in vivo monitoring of compensatory repair processes during the progression of AAAs. (Circ Cardiovasc Imaging. 2014;7:679-689.)

Key Words: aortic aneurysm, abdominal ■ elastin ■ magnetic resonance imaging

Cardiovascular disease remains the leading cause of death in Western societies and aortic disease accounts for an increasing part of the burden. The incidence of abdominal aortic aneurysms (AAAs) has substantially increased during the last 2 decades. Rupture of aortic aneurysms is the third most common cause of sudden death after myocardial infarction and stroke. There is an estimated incidence of ~5% in populations >50 years of age. More than 70,000 surgical operations and interventions are performed annually in the United States for treatment of AAAs. Several causes, including trauma, infection, and connective tissue disorders can lead to the formation of AAAs. However, most AAAs are still classified as nonspecific. Independent of the cause, rupture of the aortic wall (rupture of the elastic laminae in the tunica media) usually leads to a progressive dilatation and, if unrecognized or untreated, to potential aortic rupture with fatal consequences. The extracellular matrix (ECM) of the tunica media is the most important structural component of the aortic wall. Elastin, as the most abundant protein in the tunica media, provides the tensile strength of the arterial wall, enabling it to sustain mechanical stress resulting from high intravascular pressure and arterial pulsation. The progressive breakdown of structural proteins, in particular, medial elastin, is responsible for the inability of the aortic wall to withstand the high intraluminal hemodynamic forces. Besides elastin, different forms of collagen play an important role in maintaining aortic

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wall integrity and in the onset and progression of aortic aneurysms. Especially collagen type I and type III are important in this process. Several studies have shown that the degradation of collagen in combination with dysfunctional collagen deposition is associated with the onset, progression, and rupture of aortic aneurysms. A previous in vivo study using paramagnetic/fluorescent micellar nanoparticles functionalized with a collagen-binding protein has successfully shown the important role of this protein during the development of aortic aneurysms in a mouse model. ECM degradation and synthesis during the onset and progression of aortic aneurysms is closely linked to inflammatory processes. The influx of proinflammatory cells, including monocytes and macrophages, and the expression of proteinases, such as MMP2 and MMP9, are thought to play a central role in AAA development.

Following aortic wall rupture, local hematoma in the adventitial tissue is responsible for maintaining aortic wall integrity and preventing aortic rupture. Increasing evidence indicates that remodeling of the intramural hematoma is associated with a neovascular response and a significant synthesis of ECM proteins, including elastin. At the site of aneurysm formation, disorganized potentially newly formed elastic fibers are a recognized feature in patients. This suggests the existence of a compensatory vascular repair mechanism to strengthen aortic wall integrity and prevent aortic rupture. The balance between destruction and synthesis of ECM proteins, including elastin, is thought to be the determining factor for the development and outcome of AAs.

AAAs are usually diagnosed by screening of the aorta using ultrasound or computed tomography angiography. The majority of AAAs are small in size and do not need immediate surgical intervention. However, AAAs can enlarge over time, and their risk of rupture increases with expansion of their diameter. There is common consensus that patients with AAAs larger than 55 mm should undergo surgery. There is, however, still controversy surrounding the management of asymptomatic medium-sized AAAs (40–55 mm). No biomarker is currently available, which could help to better characterize aortic aneurysms in patients and to predict their risk of rupture.

In this study, we hypothesize that elastin-specific molecular magnetic resonance imaging (MRI) allows for the specific in vivo characterization of the aeurysmal vessel wall integrity in a small animal model of aortic aneurysm.

### Methods

#### Animals

Homozygous male ApoE-knockout mice (C57BL/6J strain background, ApoE−/−) were acquired from Charles Rivers Laboratories (Edinburgh, UK). The housing and care of the animals and all the procedures used in this study were performed in accordance with the guidelines and regulations of the United Kingdom Home Office. Eight-week-old mice were implanted with minipumps (Alzet model 2004; Durect Corp) for continuous subcutaneous infusion of angiotensin-II (Ang-II) at a dose of 1000 ng/kg/min. Mice were imaged and tissue was harvested at weeks 1, 2, 3, and 4 after Ang-II infusion (n=8 per group). The negative control mice (sham-operated group, n=6) were infused via implanted minipumps with saline for 4 weeks. Eight mice were scanned at each time point pre, post control agent (Magnevist; Bayer Healthcare AG, Berlin), and post-elastin-specific magnetic resonance molecular imaging agent (ESMA). On day 1, precontrast magnetic resonance imaging (MRI) was performed and followed by the administration of gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA; 0.2 mmol/kg) to investigate nonspecific contrast agent uptake. On day 2, 0.2 mmol/kg of ESMA was administered. Additional precontrast scans were performed in 3 mice of each group on day 2 to exclude any residual retention of Gd-DTPA in the aeurysmal aortic wall. Following the final imaging session, mice were euthanized for histology, inductively coupled plasma mass spectroscopy (ICP-MS) or electron microscopy (Figure I in the Data Supplement).

#### Anesthesia and Euthanasia

Animals were anesthetized by an intramuscular injection of a combination of Medetomidine (500 μg/kg), Midazolam (5 mg/kg), and Fentanyl (50 μg/kg). For serial imaging experiments, a reversal agent Atipamezole (2.5 mg/kg), Flumazenil (500 μg/kg), and Naloxon (1200 μg/kg) was administered. After induction of anesthesia, the animal was positioned in supine position on a 47-mm surface coil (Philips Healthcare, Best, the Netherlands).

Terminal exsanguination was performed by arterial perfusion via the abdominal aorta with PBS at a constant pressure of 100 mmHg, with outflow through the right atrium. This was followed by constant pressure perfusion in situ with 10% formalin, if vessels were embedded for histology. The suprarenal aorta was excised including the last pair of intercostals and the right renal artery.

#### In Vivo Magnetic Resonance Experiments

Imaging was performed using a 3T Philips Achieva Magnetic Resonance (MR) scanner (Philips Healthcare, Best, the Netherlands) equipped with a dedicated cardiac software package and a clinical gradient system (30 mT/m, 200 mT/m/ms). Imaging was performed in supine position with mice positioned on top of a microscope single loop surface coil (diameter=47 mm). A MR compatible heater system (Model 1025; SA Instruments Inc, Stony Brook, NY) was used to maintain the body temperature at 37°C.

For localization of the abdominal aorta and renal arteries, a low-resolution 3-dimensional (3D) gradient echo scout scan was performed in the coronal and transverse orientation using the following parameters: field-of-view (FOV)=200 mm, matrix=320, slice thickness=2 mm, TR/TE=20/5.8 ms, flip angle=30°, and slices=9. The scout scan was followed by a 2-dimensional (2D) time-of-flight (TOF) scan in the transverse orientation for visualization of the aorta. Imaging parameters included FOV=20×20×10 mm, matrix=160, in-plane spatial resolution=0.3×0.3 mm (reconstructed 0.13×0.13 mm), slice thickness=0.5 mm, TR/TE=37/7.7 ms, and flip angle=60°. From the TOF data set, a maximum intensity projection was generated to display an angiogram of the aortic arch and associated vessels and to plan the subsequent DE-MRI and T1 mapping sequences. The DE-MRI scan was preceded by a 2D Look-Locker sequence planned perpendicular to the ascending aorta, which was used to determine the optimal inversion time TI for blood signal nulling. DE-MRI followed by TI mapping sequences was started 30 to 35 minutes after contrast agent injection. Imaging parameters included FOV=30 mm, matrix=75, in-plane spatial resolution=0.4×0.4 mm, slice thickness=2 mm, TR/TE=19/8.6 ms, TR between subsequent IR pulses=1000 ms, and flip angle=10°. Imaging parameters of the IR 3D fast gradient echo DE-MRI scan used for visualization of contrast agent uptake were FOV=30 mm, matrix=300, in-plane spatial resolution=0.1×0.1, 0.5-mm slice thickness (reconstructed slice thickness=0.25 mm), 40 slices, TR/TE=28/8.2 ms, TR between subsequent IR pulses=1000 ms, and flip angle=30°.

Immediately after the DE-MRI scan, a 3D T1 mapping sequence was performed. The sequence consists of 2 inversion recovery prepared modified Look-Locker experiments (trains). Each of the 2 trains starts with a nonselective inversion pulse that uses a specific initial inversion time (12, 73 ms), followed by 8-segmented readouts for 8 individual images. The inversion times (TI) are defined as the time from the center of the preparation pulse to the acquisition of the k0 profile in k-space. Centric (low-high) k-space ordering was used.
Between the acquired k-space segments, a delay of 500 ms is used to minimize saturation effects and to allow an accurate quantification of long T1 species. Imaging parameters included FOV=18×36 mm, matrix=180×151, measured voxel size 0.2×0.2×0.5 mm, TR/TE=9.8/5.1 ms, flip angle=8°, and acquisition window=157 ms. The 2 imaging trains result in a set of 16 source images with increasing inversion times. T1 values were calculated pixel wise with a 3-parameter curve fitting procedure of the longitudinal magnetization $M(t)$ including a T1 correction.\(^{31}\)

$$M(t) = M_0 - \left( M_e + M' \right) e^{-\left(\frac{t}{1/T1} + \frac{t}{1/T1*}\right)}$$

T1 then can be calculated as follows:\(^{32}\):

$$T1 = \frac{T1*}{((M_e + M')/(M_0) - 1)}$$

$M_e$ is the equilibrium magnetization. As the longitudinal magnetization is sampled during its recovery, the relaxation process is influenced by the constant application of RF pulses resulting in the measurement of an apparent relaxation time $T1^*$ ($T1^*<T1$; $R1=1/T1$; $1/T1^* = \frac{1}{T1} + \frac{1}{T1*}$ Look-Locker) and a reduced equilibrium magnetization $M_e^*$.

After completion of scanning, mice were euthanized by dislocation of the neck and the aneurysmal aortas retrieved for ICP-MS.

**MRI Analysis**

Analysis was performed using OsiriX (OsiriX Foundation). To register the lumen and arterial wall of the aneurysmal aortas, TOF images were automatically coregistered and overlaid with high-resolution DE-MRI images. All morphometric measurements were performed on high-resolution DE-MRI images after the administration of ESMA. For signal intensity measurements, regions of interest were defined as areas of enhancement on high-resolution DE-MRI images, which colocalized with areas of aneurysmal aortic tissue (highest signal within the arterial wall). For these areas, contrast-to-noise ratio (CNR) was calculated using the following equation: CNR=(Combined vessel wall and aneurysmal aortic tissue signal–Blood signal)/Noise. Noise was determined as the standard deviation in air, anterior to the aneurysmal aorta.

**Mass Spectroscopy**

ICP-MS was performed on a subset of aneurysmal aortic tissue samples (n=3 per group). Vessel samples were digested in 70% nitric acid at 37°C overnight immediately after the last imaging session, followed by dilution with deionized water for ICP-MS analysis. A standard curve was acquired with each sample set for gadolinium concentration determination.

**Electron Microscopy**

Aneurysmal aortas were prepared by cryofixation against a liquid nitrogen-cooled metal block (n=3). Cryosections were cut at −120°C, transferred to pioloform-coated nickel grids and freeze-dried overnight. The sections were coated with a thin layer of carbon and viewed and analyzed in an electron microscope (FEI Tecnai 12), equipped with an EDAX EDS detector. Mapping was achieved with the EDAX software (EDAX).\(^{33}\)

**Histology**

Aneurysmal aortas were embedded in paraffin (n=3 per group). Sections (5 μm) were cut every 30 μm along the Aneurysmal aortas (starting from the proximal end). Selected sections were stained with hematoxylin and eosin and Miller’s Elastica van Gieson (EvG) stain.

**Aortic Aneurysm Morphometry**

The suprarenal aorta was excised including the last pair of intercostal artery and the right renal artery. Landmarks for coregistration were the left renal artery and the last pair of intercostal artery. A TOF angiogram was always acquired before planning of the DE-MRI imaging slices. Based on the 3D maximum intensity projection reconstructed from the TOF angiograms, high-resolution DE-MRI imaging slices could be planned exactly perpendicular to the course of the aorta. All morphometric analyses were made on elastin-stained sections. Morphometry was performed using ImageProPlus software (ImageProPlus, MediaCybernetics). To measure %EvG stain area per adventitial area on histological sections, the adventitia, media, intima, and lumen of the vessel (including the aneurysm) were included and the area was defined as adventitial area. The color profile of the normal elastic laminae in the media on each histological section was set as an internal reference standard. All structures within the adventitial area with this specific color profile were automatically segmented and resulting areas were recorded. The %EvG stain area per adventitial area was determined by dividing the segmented area by the overall adventitial area. Results were reported as percentage in regard to the overall adventitial area.

**Statistical Analysis**

Values are expressed as mean±SD. Treatment group values were compared with their controls using SigmaStat (Systat Software). A Student $t$ test (unpaired, 2-tailed) was applied for the comparison of continuous variables. In case of $>2$ groups, statistical comparisons were performed by ANOVA followed by the Bonferroni test. Univariate correlations were calculated using the Pearson correlation method. $P<0.05$ was considered statistically significant.

**Results**

**Assessment of AAA Areas Using an Elastin-Specific Contrast Agent**

In the control group (sham, ApoE\(^{-/-}\) mice after 4 weeks of saline infusion using osmotic mini pumps), we did not observe the formation of suprarenal aortic aneurysms (n=6, Figure 1A). The continuous infusion of Ang-II (1000 ng/kg/min), however, induced the formation of aneurysms in the suprarenal portion of the aorta (Figure 1B and 1C). This region is known to be highly susceptible to aneurysm formation in this model.\(^{15,26,34}\) Cross-sectional areas of AAAs were assessed 1, 2, 3, and 4 weeks after continuous Ang-II infusion (n=8). The in vivo MRI protocol included scans prior to administration of contrast agent (precontrast scan), after administration of Gd-DTPA (nonspecific control agent), and ESMA (Figure 1 in the Data Supplement).\(^{27}\) A significant increase in abdominal aortic cross-sectional areas was observed for the 4-week time course of Ang-II infusion (Figure 1B). Cross-sectional area measurements derived from in vivo MR scans using ESMA were compared with ex vivo area measurements on histology (EvG stain). In vivo cross-sectional area measurements after ESMA administration significantly correlated with ex vivo area measurements (Figure 1D). No significant enhancement of the aneurysmal wall was measured on scans prior to and after administration of the nonspecific control agent (Gd-DTPA).

In some mice, arterial rupture led to death through loss of blood into the abdominal cavity (n=6) prior to imaging. These mice were excluded from the study and replaced. In almost all mice after Ang-II infusion, aortic wall rupture was observed by histological examination and MRI. In 1 mouse after 1 week of Ang-II infusion and 2 mice after 3 weeks of Ang-II infusion, we did not observe aortic wall rupture. The 2 mice from the 3-week group, however, showed a significant ($P<0.05$) increase in aortic area compared with the control group.
Development of Aortic Aneurysms

The continuous Ang-II infusion resulted in the formation of aortic aneurysms with the rupture of the aortic wall (rupture of the elastic laminae in the tunica media), which could be observed at all stages of AAAs (Figures 2–4). The development of AAAs was also associated with the formation of a small or large intramural hematoma (Figures 3A7 and 4A11). The intramural hematoma with surrounding adventitia represented the only remaining vascular barrier resisting intraluminal pressure and preventing aortic rupture (Figures 3A7 and 4A11).

In Vivo Detection of the Aortic Wall Rupture Site and Monitoring of Compensatory Repair Processes

At an early stage, 1 week after Ang-II infusion, ESMA-MRI allowed the in vivo visualization of the aortic rupture site of the elastic laminae prior to the dilation of the aortic lumen (Figure 2A4–2A7). Corresponding histological sections confirmed the location of the aortic rupture site of the elastic laminae (Figure 2A6). No significant expression of elastic fibers could be observed both in vivo and ex vivo, indicating that no remodeling of the matrix has occurred yet (Figure 2A6).

Two to three weeks after continuous Ang-II infusion, an increased remodeling of the hematoma associated with an increased expression of elastic fibers was observed in vivo and ex vivo. A strong increase in elastin expression was observed especially in areas directly adjacent to the vascular lumen and in between the dissected elastic laminae (Figure 3A7). These newly formed elastic fibers seemed to form a novel elastic lamina adjacent to the lumen to sustain the intraluminal pressure and thereby reinforce the stability of the arterial wall (Figure 3A5 and 3A6). This repair process could be visualized and quantified both in vivo on ESMA-MRI and ex vivo on corresponding histological sections (Figure 3). Only few elastic fibers were observed in the peripheral areas of the hematoma and in the adventitia (Figure 3A7).

At a late stage, 4 weeks after Ang-II infusion, a further increase in elastin expression was observed in the hematoma in areas directly adjacent to the vascular lumen and in between the dissected medial elastic laminae (Figure 4A8–4A11). In areas of extraluminal hematoma next to an intact media, a strong remodeling of the hematoma by elastin was observed (Figure 4A3–4A6). A strong signal could be observed in the area of remodeling on in vivo ESMA-MRI and ex vivo on corresponding histological sections (Figure 4A3–4A6).

As already suggested by other studies,15 the outcome of aortic aneurysms may depend on the balance of ECM degradation and formation, with elastin being the main protein providing stability of the arterial wall, resisting the intra-arterial pressure and preventing rupture.

CNR and T1 Mapping

On vessel wall scans prior to administration of ESMA (precontrast) and after administration of Gd-DTPA, a low CNR and R1 (on T1 maps) in the aortic wall in all mice (sham and Ang-II infusion) were observed (Figure 5A and 5C). After administration of ESMA, a gradual and significant ($P<0.05$) increase in CNR and R1 was observed in the aneurysm aortic wall starting from 1 to 4 weeks of Ang-II infusion (Figure 5A and

Figure 1. In vivo and ex vivo assessment of abdominal aortic aneurysms cross-sectional area. A, Time-of-flight (TOF) angiogram (A1) of the suprarenal part of the abdominal aorta (aA) 4 weeks after continuous infusion of saline in a male ApoE$^{-/-}$ mouse (sham, control). The red line indicates the orientation of subsequently performed transverse MRI sequences. A nondilated aortic lumen was found on the angiogram and elastin-specific magnetic resonance molecular imaging agent (ESMA) scan (A2, A5; precontrast scan; A3, A6; Gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA) with control agent; A4, A7; ESMA scan; A2–A7 corresponding images fused with TOF with same magnification as C2–C7). No significant enhancement of the aortic wall could be measured after the administration of the nonspecific control agent (Gd-DTPA) compared with the precontrast scan. B, A significant increase in aortic cross-sectional areas was observed for the 4-week time course of angiotensin-II (Ang-II) infusion on in vivo MRI images. C, Angiogram (C1) of the suprarenal part of the aA 4 weeks after the infusion of Ang-II in an ApoE$^{-/-}$ mouse (4-week group). On the angiogram, a large suprarenal aortic aneurysm could be clearly visualized (C1). On ESMA-MRI, a significantly enlarged aortic lumen and a strong enhancement of the aortic wall was visualized (C2, C5; precontrast scan; C3, C6; Gd-DTPA scan [control agent]; C4, C7; ESMA scan; C6–C7; corresponding images fused with TOF). No significant enhancement of the aortic wall could be measured before (C2, C5) and after (C3, C6) administration of the nonspecific control agent (Gd-DTPA). D, In vivo cross-sectional area measurements after ESMA administration significantly correlated with ex vivo area measurements on histology (Elastica van Gieson [EvG] stain). Values are expressed as mean±SD. rRA indicates right renal artery.
5C). These temporal trends in CNR and R1 were in agreement (P<0.05) with quantification of %EvG stain area per adventitial area on histological sections (Figure 5B and 5D). A minor however not significant (P>0.05) progressive increase in CNR and R1 could also be observed on precontrast and Gd-DTPA scans (Figure 5A and 5C). This effect was because of the minor intrinsic T1 shortening effect of the intramural hematoma. To exclude contrast effects resulting from Gd-DTPA in the second imaging session, additional precontrast scans were performed in 3 mice of each group on day 2. No significant difference (P>0.05) in vessel wall enhancement and, therefore, no significant residual retention of Gd-DTPA were measured between precontrast scans on day 2 and precontrast scans on day 1.

In Vivo Assessment of Adventitial Tissue Remodeling Proximal to the Aortic Aneurysm

In the region proximal to the aortic aneurysm, a regular nondilated aortic lumen with intact medial elastic laminae was observed. In the control group, only a thin adventitia without significant deposition of elastin was found at this location (Figure 6A1–6A3). During the time course of Ang-II infusion, a progressive and significant increase in adventitial tissue remodeling with elastin was observed in this region with an increased expression of elastic fibers (Figure 6A9–6A11). This process resulted in a thickening of the adventitia (Figure 6A11, orange arrow). Corresponding to the increased expression of elastic fibers on histological sections (Figure 6A3, 6A7, and 6A11), an increase in aortic wall enhancement was measured on ESMA-MRI (Figure 6A2, 6A6, and 6A10). This process was quantified in vivo on ESMA-MRI. A significant increase in CNR and R1 was observed during the time course of Ang-II infusion (Figure 6B and 6C). Additionally, a significant (P<0.05) correlation between CNR and EvG stain area/adventitial area was found (Figure 6D).

Gadolinium Concentration by ICP-MS

The average concentration of gadolinium in the aneurysmal aorta wall increased substantially as disease progressed from the initial to advanced stages (n=15, Figure 7A and 7B). A significant correlation of CNR (P<0.05) and R1 (P<0.05) with ex vivo measured gadolinium concentrations (ICP-MS) was found (Figure 7A and 7B).

Electron Microscopy

To determine the gadolinium distribution within the arterial wall after ESMA administration, we acquired x-ray spectra at various locations across the arterial wall sample (n=3) and mapped the distribution of gadolinium. Colocalization of targeted gadolinium with elastic fibers was found (Figure 7C, n=3). The spatial distribution of sodium and potassium were mapped as control; however, no specific distribution pattern was observed.

Discussion

In this study, we report the in vivo characterization of AAAs using elastin-specific molecular MRI. The elastin-specific magnetic resonance molecular imaging agent (ESMA) applied allowed the noninvasive detection of the rupture site prior to aortic dilation and the subsequent in vivo monitoring of compensatory repair processes during development of AAAs. Changes in elastin composition of the arterial wall at different stages of AAA development could be readily delineated and quantified in vivo. MR signal intensity after ESMA administration significantly correlated with the %EvG stain area per adventitial area. This pattern differed markedly from that observed with the commonly used control contrast agent, Gd-DTPA, for which no significant accumulation was measured. In vivo aneurysmal CNR and R1 values after EMSA administration were in good agreement with ex vivo gadolinium concentrations as determined by ICP-MS. There are various potential applications for an elastin-specific contrast agent in the context of AAAs. Unlike existing clinical approaches, which are based on aneurysmal cross-sectional area or diameter measurements for risk evaluation, ESMA-MRI enables the specific assessment of alterations within the arterial wall at the molecular level.
In Vivo Detection of the Aortic Wall Rupture Site and Monitoring of Compensatory Repair Processes

The pathogenesis of human AAAs is not fully elucidated yet. Previous studies investigating the pathogenesis of AAAs mainly relied on tissue samples obtained during surgical interventions of end stage of AAAs. At this late stage, pathological features of the aortic wall include expression of elastic fibers, adventitial hypertrophy, and accumulation of proinflammatory cells. Limited data are available on the composition of early-stage human AAAs. An increasing amount of data are however indicating that aortic rupture (rupture of medial elastic laminae) could be the initiating event for the development AAAs in humans. Following medial rupture, which results from the destruction of elastic laminae and leads to the formation of aortic aneurysms, remodeled aneurysmal hematoma and adventitial tissue are thought to play the leading role in the stabilization of the aortic wall. The ability of aneurysmal hematoma and adventitial tissue to remodel and resist the intra-arterial pressure was suggested to be the determining factor for the potential further dilation and outcome of AAAs in animal models. There is also accumulating evidence in humans that one of the key proteins for the maturation of aortic aneurysms is elastin, as, for example, suggested by the increased expression of tropoelastin messenger RNA in human aneurysm tissue. Compensatory repair mechanisms are also thought to result in the reduction of aneurysm size after successful endovascular repair of AAAs. These observations support the hypothesis that compensatory repair mechanisms associated with a high elastin expression play a critical role in maturation and stabilization of the aneurysmal aortic wall in patients with AAAs. These observations reinforce the relevance and potential impact of our findings.

Figure 3. In vivo assessment of compensatory repair mechanisms. Angiogram of the suprarenal part of the abdominal aorta (aA; A1, A2 fusion with elastin-specific magnetic resonance molecular imaging agent [ESMA]) 3 weeks after continuous infusion of angiotensin II. At this time point, a significant increase in luminal cross-sectional areas could be observed, which was associated with the rupture of elastic laminae (magnification of A5, orange arrows). We observed a strong increase in elastin formation at the site of hematoma formation directly adjacent to the dilated vascular lumen (A3, A4). These newly formed elastic fibers at the site of hematoma formation seem to bridge the area in between the dissected medial elastic laminae (magnification A7, red arrows). This process could be clearly visualized in vivo as a strong enhancement on ESMA-MRI (magnification of A5, A6, red arrows). The undisrupted part of the former arterial wall only showed a moderate enhancement (dotted arrows, +). Few elastic fibers were observed in the peripheral areas of the hematoma and in the adventitia (magnification of A7). As the lumen of the aneurysm aorta is collapsed, it might not accurately reflect the true cross-sectional area. On precontrast (A3) and gadolinium diethylenetriamine penta-acetic acid enhanced images (A4), only minor enhancement of the arterial wall was observed. EvG indicates Elastica van Gieson; HE, hematoxylin and eosin; rRA, right renal artery; and TOF, time of flight.
In this study, the initiating event for the formation of AAAs was the rupture of the elastic laminae in the tunica media of the aorta. Comparable observations were made by other groups using this model. ESMA-MRI allowed the noninvasive detection of the rupture site as an initiating event. This observation is of high significance, as this is the first study demonstrating that it is feasible to noninvasively detect the site of the aortic rupture on a molecular level prior to the dilation of the aortic lumen or the formation of a false lumen.

Following rupture, the intramural hematoma is replaced by ECM proteins with elastin as a main component. This process is thought to be the compensatory response of the arterial wall to resist the intra-arterial pressure. We observed a strong increase in elastin formation adjacent to the vascular lumen in between the dissected elastic laminae in vivo by ESMA-MRI and ex vivo on EvG stains. In the late phase of remodeling, the intramural hematoma was almost completely replaced by ECM proteins with elastin as a main component. This process indicates the stabilization of the previously fresh hematoma. ESMA-MRI allowed the in vivo visualization and quantification of these processes.

Adventitial tissue remodeling also occurred in the area of the intact aortic wall proximal to the dilated lumen. ESMA-MRI enabled the visualization and quantification of this compensatory changes surrounding the intact tunica media. Detecting and quantifying this process in vivo could yield important information, for example, in the context of hypertension, as it is well established that the development of hypertension is associated with an increase in ECM proteins, including elastin. Previous studies have indicated that elastin synthesis could be directly stimulated by high blood pressure itself.

**Screening for and Characterization of Aortic Aneurysms**

Ultrasonography and computed tomography angiography are the most commonly used techniques for screening, initial assessment, and follow-up of patients with AAAs. For small asymptomatic AAAs (<35 mm), close follow-up is...
recommended, whereas for large AAAs (>55 mm), surgery or endovascular repair is the recognized management. In clinical practice, the diameter of AAAs is still the only recognized feature to predict risk of rupture. Although there is general agreement that large aneurysms should undergo definite treatment, there is still controversy about the management of medium-sized aneurysms (40–55 mm). This controversy reflects the limitation of diameter as a marker of risk of rupture. For the more detailed assessment of patients, a novel marker is, therefore, urgently needed. ESMA-MRI could allow the detection of the rupture site and the characterization of compensatory repair processes in the tunica media and adventitia on a molecular level. As these processes were shown to be associated with the outcome of AAAs, ESMA-MRI could provide important information for a more accurate risk stratification of this patient group.

Translational Potential of ESMA

With regard to translation into clinical applications, our approach has several advantages. All imaging was performed on a clinical 3T MRI system. Therefore, relaxation, rotational correlation, and signal properties of the contrast agent are unchanged, and imaging findings can, therefore, be directly translated to human applications. The molecular composition and size of the contrast agent are comparable to contrast agents already used in clinical practice making adverse effects substantially less likely compared with larger molecules, for example, antibodies and nanoparticles. Furthermore, the clearance of the contrast agent is comparable to approved gadolinium-based MR contrast agents allowing for early imaging after injection with minimal background signal and thus maximal target to blood contrast to noise. The high-resolution DE-MRI and the T1 mapping sequences we developed and used are directly applicable to large animal models and humans with an even higher expected accuracy and specificity due the 10 to 20 times greater arterial wall thickness and consequently higher elastin content.

Limitations

The main difference between human AAAs and those in Ang-II infused mice is the anatomic location. In humans, AAAs preferentially develop in the infrarenal aorta, whereas in ApoE−/− mice, AAAs develop in the suprarenal aorta. The exact mechanism, which results in this difference, is unknown. One potential explanation is the difference in hemodynamics and blood pressure, which is caused by altered mechanical properties of the artery as a result of regional differences in elastin composition. A further difference between the human and murine aorta is that there is a significantly higher density of smooth muscle cells and collagen in the human aorta. In mice, elastin is organized in different layers in the media. In humans, however, the highest density of elastic fibers can be found in the IEL and EEL close to the basal membrane and the adventitia. Using binding assays it could be shown that the elastin-specific molecular probe binds to elastin with the

Figure 5. Quantitative in vivo assessment of compensatory repair processes. A and B, Comparison of average contrast-to-noise ratio (CNR) values calculated from measurement on elastin-specific magnetic resonance molecular imaging agent (ESMA)-MRI (n=8 per aneurysm group, n=6 sham group). A significant and steady increase in CNR could be observed with increasing time of angiotensin-II (Ang-II) infusion. B, Scatter plots show a significant (P<0.05) correlation between in vivo CNR measurements after ESMA administration and corresponding %Elastica van Gieson (EvG) stain area per adventitial area measurements on EvG-stained sections (n=15). The signal intensity of ESMA measurements significantly correlated (R²=0.77, P<0.05) with the amount of the expression of elastic fibers in the remodeled aortic wall. C and D, Respective average R1 values over the time course of Ang-II infusion derived from T1 maps after ESMA administration and correlation with EvG-stained histological sections. Values are expressed as means±SD.
highest affinity compared with other ECM proteins in the aortic wall. The exact mechanism of binding of the molecular probe to its target protein was, however, not evaluated. Compared with other animal models, the advantage of the model we used is that no surgical intervention at the aorta is required and aneurysms develop spontaneously in response to the Ang-II infusion. In many cases, aortic aneurysms are associated with atherosclerotic vessel wall changes, and it has been,
therefore, hypothesized that aneurysms may be a consequence of atherosclerosis.\(^5\) This view has, however, been increasingly challenged in recent years. Different clinical and basic science studies have shown that the underlying pathological mechanisms differ between these 2 diseases.\(^6\) However, there might be certain common pathways.

**Conclusions**

We demonstrated that elastin-specific molecular MRI allows for the in vivo characterization of AAAs. Changes in elastin composition of the aortic wall at different stages of aortic aneurysms could be readily delineated and quantified in vivo. ESMA-MRI offers potential for the noninvasive detection of the aortic rupture site prior to the dilation of the aorta and the subsequent in vivo monitoring of compensatory repair processes during the progression of AAAs. ESMA-MRI could therefore provide important molecular information for a more accurate risk stratification of patient with AAAs.

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**Disclosures**

The MRI scanner is partly supported by Philips Healthcare. Dr Lutgens and Robinson are employees of Lantheus Medical Imaging. The contrast agent was provided by Lantheus Medical Imaging. The other authors report no conflicts.

**References**

CLINICAL PERSPECTIVE

The incidence of abdominal aortic aneurysms (AAAs) has substantially increased during the last decades. Rupture of AAAs is the third most common cause of sudden death. Most AAAs are still classified as nonspecific. AAAs are usually diagnosed by screening of the aorta using ultrasound or computed tomography angiography. The majority of AAAs are small in size and do not need immediate surgical intervention. However, AAAs can enlarge over time and their risk of rupture increases with expansion of diameter. There is common consensus that patients with AAAs >55 mm should undergo surgery. There is, however, still controversy surrounding the management of asymptomatic medium-sized AAAs (40–55 mm). No biomarker is currently available, which could help to better characterize aortic aneurysms in patients and to predict their risk of rupture. Elastin is the key protein for maintaining aortic wall tensile strength and stability. The progressive breakdown of structural proteins, in particular, medial elastin, is responsible for the inability of the aortic wall to withstand intraluminal hemodynamic forces. In this study, we demonstrate that elastin-specific molecular MRI allows for the in vivo characterization of AAAs. An elastin-specific molecular magnetic resonance probe could therefore enable the noninvasive detection of the aortic rupture site prior to the dilation of the aorta and the subsequent in vivo monitoring of compensatory repair processes during the progression of AAAs. Elastin-specific molecular magnetic resonance could, therefore, provide important molecular information for a more accurate risk stratification of patient with AAAs.
In Vivo Assessment of Aortic Aneurysm Wall Integrity Using Elastin-Specific Molecular Magnetic Resonance Imaging

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Supplemental Material

Binding of the molecular probe:

Fluorescence-based DELFIA binding assays using analogue Europium(III) (Eu(III)) complexes of the elastin specific molecular probe were performed. Briefly, proteins of study were coated in 96-well flat-bottom plates (Greiner Bio-One Ltd) and Eu(III)-complexes of the elastin specific molecular probe were used to perform binding assay. Different complex concentrations were incubated for two hours at room temperature, and the data obtained were used to calculate Binding Affinity Constant (KD) values. For reasons of technical feasibility a solid state assay was performed to assess the KD of peptides binding to elastin: Elastin (Elastin from Bovine Neck Ligament was purchased MP Biomedicals) was suspended in Eu(III)-complex probe solutions and left stirring for two hours at room temperature. The supernatant was removed and DELFIA Enhancement Solution (Perkin Elmer) was added. An aliquot of this solution was analyzed and KD values were calculated. Nine concentrations in the range 10nM–10μM were used for each assay and studies were performed in triplicates (n=3). Additionally, fluorescence intensity (Tecan Infinity 200 Pro plate reader) of each initial solution (for each concentration) was measured, allowing the determination of % binding to the protein of interest in function of the concentration. To allow a better comparison of data, the % binding for the concentration nearest the KD for each protein was reported. The % binding was calculated after two hours at room temperature and measured in the same conditions for proteins tested.

With these experiments it was shown that the elastin specific molecular probe is binding to elastin with a KD of 1.0 +/- 0.5 μM. In contrast, affinity to other extracellular matrix
proteins was significantly lower: Tropoelastin (Cellsystems) KD 9.2 +/- 0.7 μM, collagen type I (BD Biosciences) KD 7.3 +/- 1.3 μM, KD collagen type III (BD Biosciences) 6.8 +/- 1.2 μM. There was no significant binding observed to human serum albumin, which explains the favorable in vitro and in vivo stability and longer circulation times of the elastin specific molecular probe. Additionally, a 41% binding of the elastin specific probe to elastin and a 40% binding to tropoelastin was measured. For other extracellular matrix proteins a significantly lower binding was measured: 22% for collagen type I and 13% for collagen type III.
Supplementary Figure 1. Setup of animal study and time-points of in vivo imaging and ex vivo tissue analysis.

This figure demonstrates the setup of the animal experiments. All time points for in vivo imaging and ex vivo tissue analysis are indicated. As previous studies have already demonstrated,¹ medial dissection occurs during the first week after the implantation of the osmotic minipumps, which release Ang-II subcutaneously at a dose of 1000 ng · kg⁻¹ · min⁻¹. At later stages the remodeling of the aneurysmal tissue with extracellular matrix proteins, with elastin as a main component, can be observed.¹
Supplementary Figure 1

Day 0 1 week (d1, d2) 2 weeks (d1, d2) 3 weeks (d1, d2) 4 weeks (d1, d2)

Medial dissection

Aneurysmal tissue remodeling

Implantation osmotic minipumps

- d1
  - native MRI
  - Gd-DTPA MRI

- d2
  - Elastin MRI
  - Histology
  - ICP-MS
  - EM
References