

PRE-CLINICAL RESEARCH

In Vivo Characterization of a New Abdominal Aortic Aneurysm Mouse Model With Conventional and Molecular Magnetic Resonance Imaging

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Objectives

The goal of this study was to use noninvasive conventional and molecular magnetic resonance imaging (MRI) to detect and characterize abdominal aortic aneurysms (AAAs) in vivo.

Background

Collagen is an essential constituent of aneurysms. Noninvasive MRI of collagen may represent an opportunity to help detect and better characterize AAAs and initiate intervention.

Methods

We used an AAA C57BL/6 mouse model in which a combination of angiotensin II infusion and transforming growth factor- β neutralization results in AAA formation with incidence of aortic rupture. High-resolution, multisequence MRI was performed to characterize the temporal progression of an AAA. To allow molecular MRI of collagen, paramagnetic/fluorescent micellar nanoparticles functionalized with a collagen-binding protein (CNA-35) were intravenously administered. In vivo imaging results were corroborated with immunohistochemistry and confocal fluorescence microscopy.

Results

High-resolution, multisequence MRI allowed the visualization of the primary fibrotic response in the aortic wall. As the aneurysm progressed, the formation of a secondary channel or dissection was detected. Further analysis revealed a dramatic increase of the aortic diameter. Injection of CNA-35 micelles resulted in a significantly higher magnetic resonance signal enhancement in the aneurysmal wall compared with nonspecific micelles. Histological studies revealed the presence of collagen in regions of magnetic resonance signal enhancement, and confocal microscopy proved the precise co-localization of CNA-35 micelles with type I collagen. In addition, in a proof-of-concept experiment, we reported the potential of CNA-35 micelles to discriminate between stable AAA lesions and aneurysms that were likely to rapidly progress or rupture.

Conclusions

High-resolution, multisequence MRI allowed longitudinal monitoring of AAA progression while the presence of collagen was visualized by nanoparticle-enhanced MRI. (J Am Coll Cardiol 2011;58:2522–30) © 2011 by the American College of Cardiology Foundation

Abdominal aortic aneurysms (AAAs) are permanent dilations of the abdominal aorta that exceed the normal diameter by >50% and which present a life-threatening degenerative disease. They occur in 5% to 9% of the population

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over the age of 65 years and are the tenth leading cause of death in western countries (1). AAA progression is characterized by a long period of asymptomatic growth of the abdominal aorta. Once it reaches 5.5 cm, there is an increased chance of rupture, with a mortality rate >80%. Surgery and endovascular aortic repair (EVAR) are currently the only interventions for patients diagnosed with AAA. These procedures are costly and associated with high morbidity and mortality rates (2). Generally, surgery or EVAR is recommended when the AAA reaches a size of 55 mm or if the expansion rate is >1 cm/year (2). Although currently the best predictor of aneurysm expansion is the baseline size at diagnosis (3), the prognosis remains complex due to the nonlinearity and unpredictability of expansion rates (4). More specific prognostic predictors would offer the chance to better select intervention strategies for individual patients.

Different animal models have been developed in the past several years to improve the understanding of AAA pathophysiology. Recently, Wang et al. (5) introduced a model in which a combination of angiotensin II (Ang II) and anti-transforming growth factor (TGF)- β administered to C57BL/6 wild-type mice leads to AAA formation and the occurrence of fatal AAA rupture as high as 80%. Currently, this is the only animal model that displays such a relevant rate of AAA rupture, thereby enabling monitoring of the mechanisms at play in this fatal process.

The degradation of the extracellular matrix (ECM) in the medial wall is key to the formation, progression, and rupture of AAAs (6). Particularly, the turnover of collagen, an essential component of the ECM, is known to be responsible for the remodeling that occurs in the adventitia. Studies indicate that perturbations in collagen microarchitecture and networks, probably as a result of collagen degradation and inappropriate collagen deposition, may alter vessel wall response to mechanical load and lead to vessel wall failure (7). Therefore, imaging collagen in AAAs might provide valuable information about the state of aneurysm development and the identification of AAAs prone to severely progress or rupture.

In the current work, we first explored the use of multi-sequence, high-field magnetic resonance imaging (MRI) to characterize the development of AAAs in the aforementioned model. We then applied recently developed fluorescent/paramagnetic nanoparticles (8), functionalized with a collagen-specific protein (CNA-35) (9–13), to identify the presence of collagen in the aneurysmal wall using molecular MRI. Finally, in a proof-of-concept experiment, we studied the potential of CNA-35 micelles to discriminate between stable AAAs and aneurysms prone to progress and eventually rupture.

Methods

Animal model. All the procedures were approved by the Mount Sinai School of Medicine Institutional Animal Care and Use Committee. C57BL/6J male mice (The Jackson Laboratory, Bar Harbor, Maine) between the ages of 8 and 12 weeks were used. As described in more detail by Wang

et al. (5), AAAs were induced in wild-type mice by continuous infusion of Ang II at 1,000 ng/kg/min for a maximum of 28 days, along with systemic neutralization of TGF- β . Briefly, animals were anesthetized with isoflurane (4% induction, 1.5% maintenance), and mini-pumps (Alzet, model 2004 DURECT Corporation, Cupertino, California) containing 200 μ l of Ang II were implanted subcutaneously. To achieve systemic neutralization of TGF- β , intraperitoneal injections of an anti-TGF- β antibody (dose: 20 mg/kg) were performed every other day, which results in AAA formation in the suprarenal region of the aorta.

Multisequence in vivo MRI of AAA progression.

AAAs were induced in a group of mice as described above ($n = 3$). MRI scanning was performed using a 9.4-T, small-animal, vertical bore MRI scanner (Bruker Bio-Spec, Bruker, Germany). Each animal was placed in the center of a whole-body coil (35-mm inner diameter), under continuous isoflurane anesthesia, which was positioned in the scanner. The animals were connected to a respiratory rate monitor, and the flow of anesthetic gas was constantly regulated to maintain a breathing rate of 60 breaths/min. Baseline MRI scanning was followed by scans every other day until sacrifice. Each scan session started with a pilot scan with 3 orthogonal slices, followed by high-resolution, black-blood T1-weighted (T1W), T2-weighted (T2W), and proton density-weighted (PDW) spin echo imaging, of which the exact parameters are reported in the online version of this paper. The suprarenal region of the aorta encompassing 22 mm immediately superior to the right renal artery was imaged. Subsequently, a time-of-flight angiography sequence was used to visualize the blood flow and generate a 3-dimensional reconstruction of the aorta to allow visualization of vessel dilation.

Molecular MRI of collagen. AAAs were induced in wild-type mice ($n = 10$) according to the aforementioned protocol. The presence of an aneurysm was assessed using magnetic resonance (MR) angiography. CNA-35 micelles or the mutant Y175K CNA-35 micelles (12) (dose: 50 μ mol Gd/kg) were injected in the tail vein of the animals ($n = 5$ for each group). The mice were imaged pre- and 32 hours' post-injection to allow proper clearance of the micelles from the circulating blood, using a T1W spin echo sequence, with the same parameters as used for the multispectral imaging group (Online Appendix). To ensure that AAA remodeling remained minimal between pre- and

Abbreviations and Acronyms

AAA	= abdominal aortic aneurysm
AngII	= angiotensin II
CLSM	= confocal laser scanning microscopy
CME	= combined Masson elastin
ECM	= extracellular matrix
EVAR	= endovascular aortic repair
MR	= magnetic resonance
MRI	= magnetic resonance imaging
NIRF	= near-infrared fluorescence
%NSE	= normalized percentage signal enhancement
PDW	= proton density-weighted
T1W	= T1-weighted
T2W	= T2-weighted
TGF	= tumor growth factor

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