Molecular Imaging of Aortic Aneurysms

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Aortic aneurysms (AAs) are life-threatening permanent dilations of the aorta, frequently defined by a diameter of 1.5 times normal. They are subdivided anatomically into thoracic aortic aneurysms (TAAs) and abdominal aortic aneurysms (AAAs). The underlying pathogenesis differs between the 2 anatomic sites; for TAAs, the histological abnormality is medial degeneration characterized by loss of smooth muscle cells, fragmented and diminished elastic fibers, and accumulation of proteoglycans. Genetic mutations are the underlying cause of TAAs in many young or middle-aged patients. In contrast, the histopathology of AAAs is dominated by severe intimal atherosclerosis, chronic transmural inflammation, and remodeling of the elastic media. Analysis of gene expression demonstrated that AAAs and TAAs exhibit distinct patterns with most changes relative to normal aortas unique to each disease. However, several risk factors are shared between TAAs and AAAs, including smoking, hypertension, male sex, and aging. The age and sex dependence is illustrated by the prevalence of AAAs 2.9 to 4.9 cm in diameter, ranging from 1.3% in men aged 45 years ever smoked. Currently, the threshold for surgical treatment of AAs is predicated on the aneurysm diameter; for TAAs, the histological abnormality is medial degeneration characterized by loss of smooth muscle cells, fragmented and diminished elastic fibers, and accumulation of proteoglycans. Genetic mutations are the underlying cause of TAAs in many young or middle-aged patients. In contrast, the histopathology of AAAs is dominated by severe intimal atherosclerosis, chronic transmural inflammation, and remodeling of the elastic media. Analysis of gene expression demonstrated that AAAs and TAAs exhibit distinct patterns with most changes relative to normal aortas unique to each disease. However, several risk factors are shared between TAAs and AAAs, including smoking, hypertension, male sex, and aging. The age and sex dependence is illustrated by the prevalence of AAAs 2.9 to 4.9 cm in diameter, ranging from 1.3% in men aged 45 years ever smoked.1 Currently, the threshold for surgical treatment of AAs is predicated on the aneurysm diameter; for TAAs, thresholds of 5.5 to 6 cm for the ascending aorta and 6.0 to 6.5 for the descending aorta are commonly used, whereas for AAAs, the threshold is 5.0 to 5.5 cm. However, some AAs smaller than these thresholds rupture, whereas others larger than the thresholds are stable; better tools other than anatomic size to predict rupture of an individual AA are thus needed. The increasing use of endovascular repair of AAs may also impact clinical decision-making with respect to timing of repair.

The application of molecular imaging to the cardiovascular system has grown rapidly since the mid-1990s. Although the major focus has been detection of vulnerable plaque, a number of publications have used molecular probes to image AAs both in animal models and in humans. A variety of animal models for AA provide a substrate for the development of new diagnostics and therapeutics. Ranging from mice to pigs, they include a spectrum of surgically and biologically induced models. The power of molecular imaging to perform longitudinal studies has facilitated studies on the rate of AA development and offers the potential for new methods to assess AA status based on function rather than simply anatomic size. Functional imaging could help to predict which patients are unstable and will need surgery sooner or can be treated medically rather than surgically. In addition, the capacity for longitudinal imaging of functional biology in animal models of AA can be expected to facilitate the screening of novel treatments, whereas monitoring biological activity in patients may provide information on whether a specific therapy is working much more rapidly than simply measuring the change in AA size as a function of time, facilitating personalized treatment tailored to the individual patient. This review summarizes recent progress in the application of molecular imaging to AA in animal models and patients.

Animal Studies

A number of different targeting strategies have been used for molecular imaging of AAs. This section addresses the use of tracers focused on the various biological targets offered by AAs in animal models.

Extracellular Matrix

Turnover of the extracellular matrix plays a major role in AA development, making extracellular matrix components an attractive target for molecular imaging. Klink et al took advantage of a recently described mouse model of AAA
using a combination of angiotensin II infusion and transforming growth factor β neutralization to assess the use of nanoparticles (NPs) functionalized with a collagen-specific protein, CNA-35. The targeting ligand is derived from 2 domains of a collagen adhesion protein derived from Staphylococcus aureus. Intravenous injection of gadolinium-containing NP targeted with CAN-35 resulted in significantly greater T1-weighted signal enhancement in the aneurysmal wall compared with nonspecific NP, and the CNA-35 NPs were shown histologically to colocalize with Type 1 collagen.

In a proof-of-concept experiment, animals were imaged at Days 5 and 15 after induction of AAA, and images correlated with pathology (Figure 1). Higher uptake of CNA-35 NP correlated with stable Stage II aneurysms with high collagen uptake, whereas ruptured Stage IV aneurysms showed little uptake and low collagen content.17

Matrix Metalloproteases (MMPs) are overexpressed in both TAAs and AAAs and contribute to extracellular matrix degradation and aneurysm progression. Bazeli et al18 used P947, a broad-spectrum MMP inhibitor labeled with gadolinium through a chelator, to target MMPs in expanding AAAs in rat aortas perfused with elastase. Uptake of the targeted chelate into the aortic wall was shown by MRI to be significantly greater than for a scrambled targeting peptide or nontargeted Gd-DOTA. The area of contrast enhancement colocalized with MMP activity shown by in situ zymography.19 Sheh et al19 used an enzyme-activated optical imaging probe and intravital surface reflectance imaging to study the relationship between MMP activity and AAA growth. They found a linear relationship between MMP activity and optical signal. They also demonstrated suppression of MMP activity by daily oral administration of the MMP inhibitor doxycycline using endovascular imaging with the optical probe.19

Protease-activated near-infrared fluorescence probes have also been used to image TAAs in conjunction with multimodal imaging using fluorescence molecular tomography and CT coregistration.20 In a mouse model of reduced expression of the extracellular matrix protein fibulin 4, Kaijzel et al found graded increases in fluorescence molecular tomography signal within aneurysmal lesions from control mice to heterozygous fibulin-4R/− and homozygous fibulin-4/or mice (Figure 2). Increased MMP activity was detectable before increase in vessel size. Ex vivo zymography confirmed a similar graded increase in MMP activity.20

Inflammatory Cells

The infiltration of macrophages and monocytes into the vessel wall plays an important role in the progression of both TAAs and AAAs.21,22 Positron emission tomography in conjunction with macrophage-targeted iron oxide NP-labeled with 18F-fluorine permits detection of macrophages and monocytes with very high sensitivity. Nahrendorf et al23 studied an experimental model of aortic aneurysms consisting of apoE−/− mice infused with angiotensin II, which resulted in both TAAs and AAAs, to address the relationship between inflammation and aneurysm growth. They found that uptake of NP was significantly greater in aneurysms compared with wild-type aorta (Figure 3A–B). The number of macrophages and monocytes was increased >20-fold in the aneurysmal aortas of apoE−/− mice relative to wild-type, and the profile was dominated by proinflammatory Ly6Chigh monocytes rather than the resident macrophages predominantly seen in wild-type aortas (Figure 3C–D). They also found that the positron emission tomography signal of aneurysms imaged at 7 days predicted the rate of expansion; aneurysms with low uptake showed little expansion over the subsequent 3 weeks, whereas high nanoparticle uptake was associated with significant expansion.23

Integrins and Receptors

Both αβ3 integrin and vascular endothelial growth factor receptor are upregulated on neoangiogenic vascular endothelial cells and on inflammatory macrophages. Kitigawa et al24 used nanoparticles made from human ferritin nanocages and conjugated with Arg-Gly-Asp peptide to target the αβ3 integrin and image experimental AAAs in apo E−/− mice treated with angiotensin II. Using in situ and ex vivo fluorescence imaging after intravenous administration of NP labeled with the fluorescent dye Cy5.5, they demonstrated increased uptake of Arg-Gly-Asp peptide-targeted relative to nontargeted NP; by histology, they showed that the targeted NPs were colocalized both with macrophages and with neoangiogenesis.24

Tedesco et al25 used an engineered single-chain vascular endothelial growth factor homodimer labeled with Cy5.5 to
target the vascular endothelial growth factor receptor in the mouse angiotensin II infusion model. Using near-infrared fluorescent imaging, they showed that signal intensity was increased in aneurysmal aorta relative to remote uninvolved segments or vessels in control mice. Furthermore, signal intensity increased as a function of vessel diameter in aneurysmal segments.\(^\text{25}\)

**Thrombus**

The presence of intraluminal thrombus is a very common feature in AAAs with reported frequencies ranging from 75% to 98%.\(^\text{26,27}\) Intraluminal thrombi are associated with weakening of the arterial wall and rupture of AAAs, possibly through increased proteolysis and infiltration of inflammatory cells.\(^\text{28}\) Intraluminal thrombus thickness in patients undergoing elective repair for AAAs was correlated with activity of MMP-9 and the concentration of tissue inhibitor of matrix metalloprotease 1 in the adjacent aneurysm wall.\(^\text{29}\) The thrombus is biologically active, undergoing constant renewal at the luminal interface.\(^\text{30}\) Distal embolization can also occur, although the frequency is unclear, with reported incidence varying from 3% to 29%.\(^\text{31}\) One of the earliest uses of molecular imaging to image AAAs used \(^{99m}\)Tc-Annexin-V to target platelet activation and consequent exposure of phosphatidylserine at the interface with circulating blood. Using the elastase-perfused rat model and single photon emission CT, Sarda-Mantel et al\(^\text{32}\) showed a 5-fold increase in target-to-background ratio for aneurysms relative to normal vessels.
in control rats. Ex vivo studies on human thrombi excised during surgery for AAA repair showed a similar pattern of annexin labeling at areas where activated platelets and leukocytes accumulate. The P-selectin-specific targeting agent fucoidan, a sulfated polysaccharide derived from brown seaweed, has also been used to image thrombus in AAA. Rouzet et al.33 showed that 99mTc-fucoidan detected thrombi in elastase-treated rats with a median target-to-background ratio of 3.6.

**Human Studies**

Studies performed in patients so far have been restricted to AAAs and can be broken down into 2 groups: first, the use of small and ultrasmall iron oxide nanoparticles targeted to inflammatory macrophages and monocytes infiltrating the AAA in conjunction with MRI; and second, the use of 18F-fluorodeoxyglucose (FDG) as a marker of inflammation in conjunction with positron emission tomography.

**Figure 3.** Imaging in mice with early-stage aneurysms undergoing 7 days of angiotensin (Ang) II administration. **A,** Positron emission tomography (PET) signals from aneurysms and from wild-type mice. **B,** Representative PET-CT images of a nascent AA. Liver signal. **C,** Flow cytometric analysis of leukocytes in the aorta of wild-type mice (left 2 plots) and apoE−/− mice (right 2 plots) after 7 days of Ang II administration. The gated regions in the left plots for each mouse type depict the monocyte/macrophage population, which were further analyzed for expression of the monocyte surface marker Ly-6-C and the macrophage marker F4/80 (plots on the right). L1 indicates lineage antigens (CD90/B220/CD49b/NK1.1/Ly-6G). Numbers contained within the gated regions of the plots (left plots) indicate the percentage of living cells (macrophages/monocytes). Numbers in the quadrants (right plots) indicate the percentage of monocytes/macrophages in each gate. **D,** Serial CT angiography after PET-CT on Day 7 in Ang II-treated apoE−/− mice. Left panel shows the diameter of aneurysms with low PET signal; right panel shows diameter of aneurysms with high signal. Reprinted from Nahrendorf et al.23 with permission from Wolters Kluwer Health. AA indicates aortic aneurysm.

**Figure 4.** Top. Color maps (A–C) showing representative abdominal aortic aneurysm (AAA) slices from patients in each of the 3 groups alongside the corresponding T2-weighted anatomic images (D–F). The color scale represents the magnitude of the change in T2* value with blue indicating minimal change and red indicating a large change in T2* value. A distinctive pattern is seen for each patient group: (A) Group 1 shows a large change in T2* value only in the periluminal area; (B) Group 2, diffuse patchy changes in T2* throughout the intraluminal thrombus but no distinct focal area of USPIO uptake affecting the aortic wall; and (C) Group 3, discrete focal area of USPIO uptake involving the wall of the AAA that is distinct from the periluminal region. This patient subsequently died suddenly from presumed ruptured AAA. Bottom, Relationship of diameter and growth rate with patient group. Initial aneurysm diameters (open bars) are similar for the 3 groups, but the aneurysm growth rates (solid bars) are higher for patients in group 3 (0.66 cm/year) compared with those in Groups 1 (0.22 cm/year) and 2 (0.24 cm/year). Reprinted from Richards et al.34 with permission from Wolters Kluwer Health. USPIO indicates ultrasmall paramagnetic iron oxide.
Iron Oxide Nanoparticles

Richards et al. used uptake of ultrasmall paramagnetic iron oxide (USPIO) nanoparticles to assess whether inflammation correlated with AAA growth in stable patients with asymptomatic AAAs from 4 to 6.6 cm. Using T2*-weighted MRI, they found 3 patterns of USPIO uptake: periluminal uptake only, diffuse patchy uptake within the intraluminal thrombus, and discrete focal uptake distinct from the periluminal region (Figure 4). Although the initial diameter of the aneurysms was similar for all 3 groups, the annual growth rate for patients with distinct mural uptake was 3-fold greater than for the other 2 groups of patients. Uptake of USPIO into AAAs, resulting in decreases in T2 and T2* times, was also demonstrated by Sadat et al. in a small feasibility study.

Nchimi et al. used SPIO nanoparticles to assess leukocyte phagocytic activity in patients undergoing surgery for AAA. Injection of SPIO resulted in decreased T2*-weighted signal intensity in the luminal sublayer and thrombus. In some cases, the thrombus had a multilayered appearance, and this was correlated with higher MMP-9 activity in the thrombus. Semiquantitative analysis of the decrease in thrombus T2*-weighted signal showed a correlation with levels of cells positive for CD68 and for CD66b and also with tissue levels of MMP-9 activity in the thrombus.

FDG

FDG is taken up by inflammatory cells such as monocytes and leukocytes in an insulin-insensitive manner. Consequently, FDG uptake in the fasted state has been proposed as a marker of risk for AAA progression and rupture with higher FDG uptake associated with inflammation, aortic wall instability, and clinical symptoms. Elevated levels of FDG uptake have also been associated with regions of wall stress in both TAAs and AAAs. However, others have shown that FDG uptake is low in asymptomatic AAAs with a diameter close to surgical indications (mean diameter, 4.9 cm), perhaps reflecting decreased cell density in large AAAs. Similarly, Kotze et al. found an inverse relationship between FDG uptake and future growth rate of AAAs. The potential role for FDG and positron emission tomography in monitoring AAAs is thus unclear at present.

Summary

Animal studies have demonstrated that a wide range of tracers aimed at different pathophysiological elements can be used to detect AAs. These molecular imaging probes have the potential to provide complementary functional information that goes beyond the anatomic information obtained through diagnostic echocardiography or CT studies. Although anatomic imaging cannot predict future expansion of aneurysms, the functional information provided by molecular imaging agents may be able to predict future events. For some agents, for example CNA-35 targeting of collagen and 18F-labeled NP targeted to macrophages, proof-of-principle studies have confirmed that molecular imaging can provide information on AA functional status. For the collagen-targeted tracer, stable Stage II AAAs have a higher signal than ruptured Stage IV AAAs, reflecting the less-compromised status of the extracellular matrix. For 18F-NP, low uptake was associated with low growth, whereas AAs that took up high levels of tracer expanded rapidly. More extensive studies, including experiments in additional models of AA, will be needed to confirm the potential prognostic value of these and other molecular imaging strategies.

Translation to Human Studies

Each imaging technology used for molecular imaging has strengths and weaknesses related to factors such as spatial resolution, sensitivity, and depth of penetration, and these factors have been the subject of a recent comprehensive review. In some cases, the imaging modalities used to study AAs in animal models, for example MR, positron emission tomography, and single photon emission ECT, could be readily translated toward clinical application. For positron emission tomography and single photon emission CT, the lower spatial resolution can make image interpretation problematic, and so they are likely to be used in hybrid imaging with either CT or MRI to provide anatomic information. This allows accurate localization of the molecular probe and quantification of tracer in the target. The rapid expansion in availability of positron emission tomography/CT and single photon emission CT/CT hybrid systems makes this increasingly feasible, whereas hybrid positron emission tomography/MRI systems are just starting to become available commercially. Quantification of tracer uptake will be important for longitudinal studies and for comparisons between patients, and it also been proposed that quantification of tracer uptake, for example by standardized uptake value, could potentially be used to set thresholds for when a patient should undergo repair.

For tracers detected using optical methods, the limited depth penetration through tissue would preclude external detection in humans and require a catheter-based approach. Although catheters have been developed for intravascular imaging of vulnerable plaque, the invasiveness of the approach may not lend itself readily to screening and assessment of AAs. However, the use of optical methods in animal models can provide a relatively high throughput and inexpensive modality for use in longitudinal studies for drug development and testing. Hybrid imaging with anatomic imaging method such as CT can also help to overcome the limited spatial resolution of optical imaging. Enzyme-activatable probes can also provide high signal-to-noise ratios because the nonactivated probe gives no signal.

Some probes currently being used for optical imaging could be readily adapted to other imaging modalities, allowing transfer of the probe to imaging large animals and humans; for example, the human ferritin nanocages used by Kitigawa et al to target αvβ3 integrin can readily incorporate iron oxide for MRI, and single-chain vascular endothelial growth factor homodimer for vascular endothelial growth factor receptor imaging should be amenable to radiolabeling for positron emission tomographic imaging. Pros and cons for different molecular imaging agents, and their potential for clinical translation, are summarized in the Table.
Initial studies in humans show promise for the potential of molecular imaging in patient stratification, particularly the pilot study from Richards et al demonstrating that discrete focal USPIO uptake in areas distinct from the periluminal region was associated with high rates of aneurysm expansion. Patients with this pattern of USPIO uptake might thus be candidates for earlier surgical or endovascular intervention, whereas patients with only periluminal uptake, or diffuse patchy uptake within the intraluminal thrombus, might be candidates for continued monitoring and medical treatment. Larger prospective studies will be needed to determine whether this promising finding holds true. Molecular imaging agents could also potentially help in assessing efficacy of drugs using molecular imaging signals such as decreased MMP activity or altered USPIO uptake as a surrogate end point to predict whether a patient is responding to treatment. The demonstration of the efficacy of doxycycline in reducing MMP activity in mice AAAs illustrates this potential role.19 Other questions include whether there is potentially a role for molecular imaging in understanding the increased risk for AAAs in smokers.45

The etiology of TAAs and AAAs differs; AAAs are generally the result of chronic inflammatory atherosclerosis and are associated with diffuse diathesis,46 whereas TAAs reflect medial degeneration. This raises the question of whether different molecular imaging agents will be needed for the 2 disease entities. However, increased uptake of inflammatory cells such as macrophages and monocytes has been demonstrated in TAA as well as AAA,22 and elevated MMP activity has also been demonstrated. This is supported by the studies in angiotensin II-treated apo E−/− mice, where 18F-NP uptake was observed in both TAAs and AAAs,20 and in mice with decreased fibulin 4 expression where graded increases in MMP activity were found as fibulin 4 decreased.20 Thus, although human molecular imaging studies have so far been largely restricted to patients with AAAs, many of the imaging agents under development might be expected to be applicable to TAAs as well as AAAs. However, because animal models do not necessarily recapitulate human disease fully, this will need to be confirmed in clinical studies.

In summary, initial studies in animals and humans have shown promise for the molecular imaging of AAs and potentially for discriminating those at high risk for rupture from stable aneurysms.

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