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Molecular Imaging of High-Risk Atherosclerotic Plaques: Is It Clinically Translatable?

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ABSTRACT

The explosive epidemics of diabetes and obesity as well as an aging population have led to cardiovascular diseases as the leading cause of world-wide morbidity and mortality beyond cancer. The recent introduction of drug-eluting stents and medications such as statins, dual anti-platelet therapy, and angiotensin converting enzyme inhibitors has dramatically improved clinical outcomes in patients with cardiovascular diseases. However, mortality is still increasing despite state-of-the-art therapeutics, as current diagnostic and therapeutic strategies against cardiovascular disease center on "locking the barn door after the horse has been stolen". Novel diagnostic solutions that identify individuals at risk before the disease is overt are needs. Imaging approaches that visualize molecular targets rather than anatomical structures aim to illuminate vital molecular and cellular aspects of atherosclerosis biology *in vivo*. Recent technological advances in small animal imaging systems and dedicated targeted/activatable molecular imaging probes have positioned molecular imaging to greatly impact atherosclerosis imaging in the next decade. However, several issues must be addressed before its clinical translation. **(Korean Circ J 2011;41: 497-502)**

KEY WORDS: Molecular imaging; Atherosclerosis; Primary prevention.

Introduction

Percutaneous coronary intervention (PCI) reduces the incidence of death and myocardial infarction (MI) in patients with acute coronary syndrome who harbor vulnerable atherosclerotic plaques in coronary arteries.[1-3] However, PCI as initial management fails to reduce the risk of death, MI, or other major cardiovascular events when added to optimal medical therapy in those with stable coronary artery disease who have cool-down coronary plaques with lower probability of eruption but anatomically flow-limiting stenoses.[4] Furthermore, autopsy findings show that the majority of vulnerable plaques are usually <50% diameter stenosis.[5] In this regard, imaging tools that sense molecular and cellular processes within atherosclerotic plaques may be a better and/or complimentary tool than coronary lumenography assessing anatomical severity. Molecular imaging of coronary or cerebral arteries in addition to a current systemic risk evaluation such as the Framingham risk score provide a better regional and systematic ischemic event-risk portfolio.

The world is about to experience a demographic revolution. First, there is an exponential increase in patients with obesity and diabetes. Second, the world will become a super-aged society within the next two decades. Thus we can expect a greater prevalence of cardiovascular disease and greater cardiovascular mortality. In fact, cardiovascular disease is the number one killer in the world, despite state-of-the-art diagnostics and therapeutics. These conditions suggest that we must shift the mainstream of translational cardiovascular research to prior recognition of plaque rupture before patients develop acute coronary syndrome and sudden death.

Numerous molecular imaging approaches, which reveal the underlying molecular or cellular basis of atherosclerosis, have been introduced into the field of cardiovascular research in the past 10 years, using small animal imaging systems and dedicated targeted/activatable molecular imaging probes.[6,7] However, few successes in small-animal imaging of

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high-risk atherosclerotic plaques have translated clinically. In this review, we will focus on the clinical translatability of molecular imaging of atherosclerosis. First, ideal targets for imaging high-risk atherosclerotic plaques will be briefly discussed. Next, we will present which modality is most closely associated with clinical translation in the near future while considering previous validation in humans, and potential safety concerns regarding the use of molecular imaging probes.

Traditional Imaging Targets

Molecular imaging has evolved with the introduction of a variety of contrast agents that target specific cellular or molecular processes of disease relevance. In accordance with the imaging modalities and techniques being developed, contrast agents for tracking potentially important components of atherosclerotic disease have targeted various stages of atherogenesis (Fig. 1). For the past decade, imaging probes targeting adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), oxidized low density lipoprotein (LDL), apoptosis, proteases such as matrix metalloproteinases (MMPs), cathepsins or cysteine protease, and macrophages have been extensively applied to detect plaque inflammation or vulnerability.[8] First, the advantages and disadvantages of several traditional molecular imaging agents will be discussed. Then, new emerging molecular targets will be presented. Target selection is crucial for success, because most imaging targets are located in deep organs and are frequently present at very low concentrations.

Macrophages as an imaging target
Because macrophages are involved in all stages of athero-

sclerosis, including foam cell formation, plaque progression, and, ultimately, plaque disruption and thrombus formation,[9] inflammatory cells residing in plaque such as macrophages and foam cells are excellent imaging targets. Small-animal aortic magnetic resonance imaging (MRI) combined with dextranated ultrasmall superparamagnetic iron oxide nanoparticles (USPIO) has been most frequently employed to visualize atheroma-related macrophages.[10] USPIOs are engulfed by macrophages *in vivo*, followed by a detectable decrease in the T2/T2* signal in proportion to the degree of atherosclerotic plaque inflammation, as shown in human studies.[11] Our lab has also successfully imaged cellular inflammation in murine atherosclerosis using pegylated, silica-coated iron oxide nanoparticles (Fig. 2).[12] Recently, ^{18}F-fluorodeoxyglucose (FDG) positron-emission tomography (PET) has emerged as a surrogate imaging biomarker to report plaque macrophage activity and has been used to monitor anti-atherosclerosis therapy (Fig. 3).[13)14] FDG-PET will be promising when successfully extending its application into the prediction of plaque rupture and clinical events. Although macrophages are an ideal imaging target in mouse models of atherosclerosis, major differences in lesion composition between mice and humans are apparent.[15] Although inflammation is the most abundant plaque component in mice, it constitutes only 2-5% of total lesion volume in humans.[16] Currently, the most frequently used mouse models of atherosclerosis are apoE^{-/-} mice or LDL receptor-deficient mice, similar to the human model of familial hypercholesterolemia, a rare form of human atherosclerosis. These mice models develop atheromas mainly composed of foam cells and proliferating smooth muscle cells without a necrotic core or fibrous cap, typical of vulnerable human atherosclerotic plaques. Thus, macrophages may not
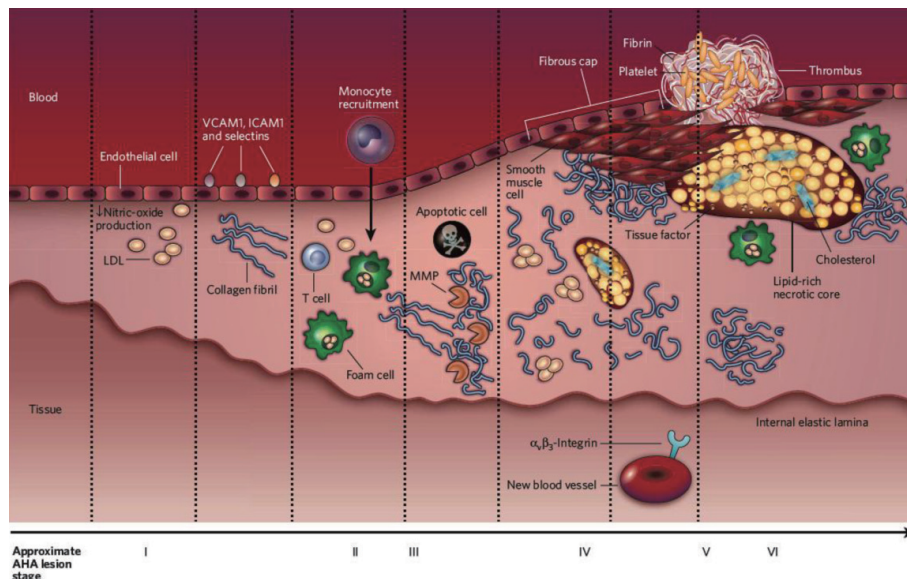
Fig. 1. Development of an atherosclerotic lesion and specific imaging targets at different stage of atherogenesis. The main cellular or molecular processes involved in each stage of atherosclerosis are presented. Potential molecular imaging targets at different stages are also listed.[8]
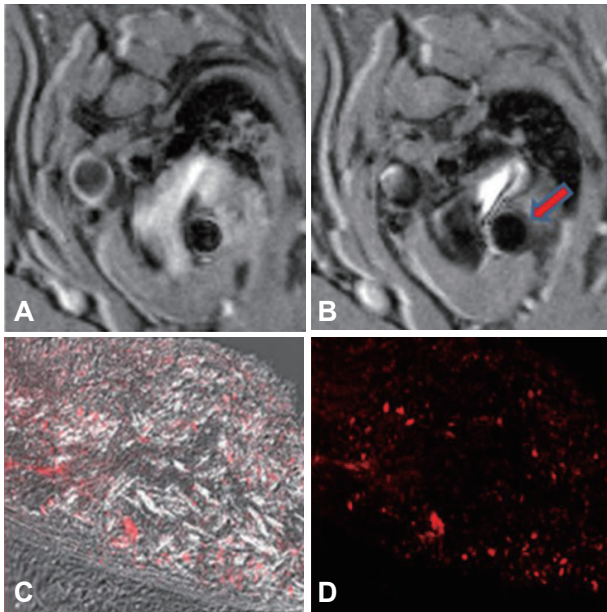
Fig. 2. Upper right panel (B) shows a dark signal in the wall of an apoE^{-/-} mouse aorta after an injection of silica-coated iron oxide nanoparticles compared with a precontrast MRI (A). Confocal microscopy shows accumulation of iron oxide nanoparticles in the aorta of a 30-week-old apoE^{-/-} mouse. Most nanoparticles are noted in the macrophage-rich plaque area (C and D).
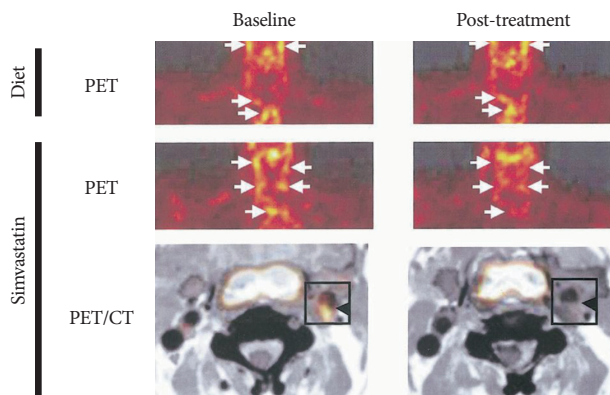
Fig. 3. Effect of simvastatin on fluorodeoxyglucose (FDG) uptake into atherosclerotic plaques. Diet alone does not have an effect on FDG uptake into carotid arteries. In contrast, simvastatin significantly attenuated FDG uptake into carotid arteries.[9]

be a good candidate for molecular imaging of high-risk atherosclerotic plaques.

Protease as an imaging target

After recruitment, macrophages scavenge lipids, secrete cytokines that further amplify inflammation, and produce proteases such as MMPs and cathepsins. In concert with the phagocytic function of inflammatory cells, proteases destabilize plaque by damaging the extracellular matrix and thinning the fibrous cap.[17] Thus , augmented protease activity within the fibrous cap is a key feature of high-risk plaques.[18] Proteases are targeted with two major strategies. Nuclear imaging probes, which could be used for noninvasive coronary imaging, are based on small-molecule protease inhibitors. An al-

ternative approach uses optical imaging agents, adopting a quenching-dequenching strategy to generate a high signal-to-noise ratio *in vivo*[19] The enzymes cleave defined peptide sequences, liberate the attached fluorochromes, and, thereby, render them fluorescent. Thus, activatable optical imaging agents have a low background fluorescence signal, in contrast with traditional targeted near-infrared fluorescence (NIRF) agents. Another advantage of activatable imaging agents is the potential to image and quantify protease activity, rather than its presence within plaques. However, targeted nuclear imaging probes need further validation for clinical translation. As in the molecular imaging of oncology, it is still unclear whether protease-targeted PET imaging can replace FDG-PET in patients with atherosclerosis. Activatable NIRF agents together with clinical type intravascular NIRF-sensing catheters can be used to detect protease activity in human coronary arteries.[20] Additionally, combining an NIRF-sensing catheter with intravascular ultrasound or optical coherence tomography will strengthen its clinical translation.[21]

Promising Imaging Targets

Apoptosis

A key element of vulnerable atherosclerotic plaques is a large necrotic core. Necrotic core is mainly formed by the combination of apoptosis of advanced lesional macrophages and defective efferocytosis in advanced plaques.[22] Prolonged endoplasmic reticulum stress and other factors such as oxidative stress and death receptor activation provoke apoptosis in macrophage foam cells.[23] These apoptotic cells are not effectively cleared by macrophages, due to defective efferocytosis in advanced atherosclerotic plaques, leading to secondary cellular necrosis. Secondary necrosis over time contributes to the formation and growth of a necrotic core.[24] Thus, apoptosis is a fascinating imaging target for plaque vulnerability. Indeed, a number of apoptosis imaging approaches have been implemented and have been applied in humans.[25] Although [99m]TC- annexin A5 imaging of inflamed carotid plaques is clinically feasible, its low resolution and specificity may be a hurdle to overcome, in addition to imaging difficulties of the coronary tree such as small plaque size and cardiac and respiratory motion.

Neoangiogenesis

The developmental stage of a fibroatheroma is consistently associated with centripetal neoangiogenesis from the adventitia toward the plaque.[26] An intraplaque hemorrhage frequently occurs, because neo-capillaries in atheromas are immature and leaky, leading to sudden expansion of the necrotic core and increasing plaque vulnerability.[27] Thus, intraplaque neovascularization and hemorrhage are postulated to play a major role in plaque progression, leukocyte infiltration, and

high-risk plaque formation. Molecular imaging agents for plaque neoangiognesis usually target αVβ3-integrin, a key mediator of angiogenesis. ^{18}F-RGD is a promising peptide tracer for PET imaging of intraplaque neovascularization, which binds with high affinity to αVβ3-integrin. ^{18}F-RGD has been extensively validated for use in oncology. However, the utility of ^{18}F-RGD PET for imaging vascular inflammation and plaque angiogenesis has not been completely clarified. In-depth translational research will clarify the clinical feasibility of ^{18}F-RGD. Recently, we verified the efficacy of apoptosis and neoangiogenesis as potential targets of clinical molecular imaging in the carotid atheromata of apoE^{-/-} mice using intravital two-photon microscopy (Fig. 4).

# Considerations for Clinically Translating Atherosclerosis Molecular Imaging

Several issues must be considered to translate molecular atherosclerosis imaging for clinical use. First, it must be determined which modality is the best fit for near-term clinical translation Second, we have limited knowledge on the fate of vulnerable atherosclerotic plaques. It is still not completely understood how a stable plaque becomes a high-risk plaque that ultimately ruptures and creates occlusive or nonocclusive thrombi in the coronary arteries. It is also yet to be investigated whether plaque vulnerability is short-lived or fairly long-lasting. How many vulnerable plaques exist in an individual patient? Will all vulnerable plaques lead to acute coronary syndrome or will a vulnerable plaque with particular characteristics be mainly responsible for the event? Because of the lack of a typical animal model with the human form of vulnerable atherosclerotic plaques, the natural history of plaque progression and the fate of vulnerable plaques are poorly understood.[15] Thus, once a molecular imaging tool is available in the clinic, there will be significant progress in clarifying the life history of plaques, but significant amounts of clinical data using molecular imaging tools will be needed to adopt routine imaging.

Recent advances in small-animal imaging technologies, such as fluorescence imaging, bioluminescence imaging, ultrasound, micro-PET, micro-single photon emission computed tomography (SPECT), micro-computed tomography (CT), and high-field (4.7T or 9.4T) small-animal MRI as well as the availability of dedicated imaging probes targeting molecular or cellular processes have positioned molecular imaging to greatly impact atherosclerosis imaging in the next decade. Clinical translation of these advances of small animal atherosclerosis molecular imaging is greatly anticipated with the introduction of human imaging platforms or imaging probes. However, several factors must be considered for imaging coronary plaques. The coronary arteries are usually 2.5-3.5 mm in diameter; thus, their plaques are too small to be imaged well enough to show molecular signals in addition to anatomy. In addition, coronary plaques are not in a static condition but move quickly with the heart and respiration. All of these conditions require imaging tools with high spatial and temporal resolution. In this regard, MRI shows promise for molecular imaging of vulnerable plaques in coronary arteries. Indeed, many investigators have explored the applicability of molecular MRI for atherosclerosis to detect macrophage activity, apoptosis, and adhesion molecules within plaques, mostly using gadolinium chelates (T1 agents) or iron oxide nanoparticles (T2/T2* agent). However, MRI must overcome the major obstacle of lower imaging sensitivity compared with that of PET, which requires only several nanograms of PET agent for molecular imaging (Table 1). However, PET has intrinsic weaknesses for applications in cardiovascular molecular imaging because of its limited spatial resolution (clinically, 4-10 mm; small-animal imaging, 1-2 mm). We will consider this imaging modality issue in detail in the next section.

Magnetic resonance imaging

MRI as a molecular imaging modality has many strengths; its remarkable spatial resolution, of which 3T MRI provides 10 to 100 μm resolution, lack of radiation exposure, and its
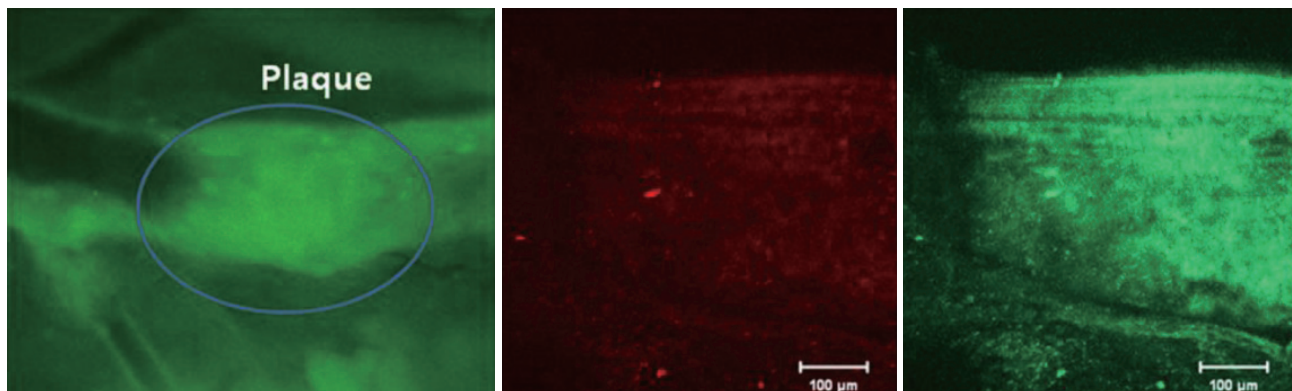
Fig. 4. High-resolution *in vivo* fluorescence molecular imaging of intraplaque neoangiogenesis and apoptosis in carotid atheroma of apoE^{-/-} mice. After injecting angiogenesis- and apoptosis-targeting molecular imaging agents, intravital two-photon microscopy of carotid atheromata in a 34 week-old apoE^{-/-} mouse revealed discrete plaque-specific neoangiogenesis (middle) and apoptosis (right).

Table 1. Characteristics of molecular imaging modalities

| Imaging modality	Spatial resolution (mm)	Imaging sensitivity (mol/L) probe
Fluorescence imaging	≤1	10^{-10}–10^{-12}
PET	4-10 (clinical PET) 1-2 (microPET)	10^{-11}–10^{-12}
SPECT	7-15 (clinical SPECT) 0.5-2 (microSPECT)	10^{-10}–10^{-11}
MRI	0.5-1.5 (1.5T MRI) 0.01-0.1 (small animal MRI)	10^{-3}–10^{-5}
CT	0.5-2 (clinical CT) 0.02-0.3 (microCT)	10^{-2}–10^{-3}

PET: positron emission tomography, SPECT: single photon emission computed tomography, MRI: magnetic resonance imaging, CT: computed tomography

unique ability to combine anatomical, functional, and molecular information. In contrast, the low imaging sensitivity of MRI is a fundamental weakness for clinical translation. Low imaging sensitivity means that MRI requires a large volume of agent for molecular imaging, which is associated with toxicity. Molecular MRI adopts specialized imaging agents that have a relatively larger diameter than conventional gadolinum chelate, because a longer circulation time is mandatory to bind to tissue targets, in addition to the T1 or T2/T2* shortening effect. Thus, most imaging agents for molecular MRI are nanometer-sized.

However, knowledge is incomplete regarding nanoparticle toxicity, biodistribution, excretion, and pharmacokinetics. The Food and Drug Administration (FDA) applies the same degree of regulatory oversight for nanoparticulate imaging agents as new drugs. In the near term, we are not ready to intravenously inject nanoparticulate molecular imaging agents into humans. Further efforts are required to realize molecular MRI as routine practice

Positron-emission tomography

Currently, PET and SPECT are the most translatable noninvasive molecular imaging platforms because of the availability of existing clinical scanners, the availability of versatile radiochemistry options, and the ongoing development of new imaging agents, which only need to be administered in a small, nonpharmacological dose (nanograms) for highly sensitive imaging. Although PET is the most sensitive and quantitative modality, it has limited spatial resolution and, unfortunately, physical constraints make it unlikely that PET spatial resolution will improve further. Thus, a combination of SPECT/PET with CT images or PET with MRI is being studied to further enhance anatomic localization. We identified the superiority of FDG PET/CT of carotid arteries compared to ultrasound for detecting plaque inflammation (Fig. 5). However, FDG PET/CT has several inherent weaknesses for use
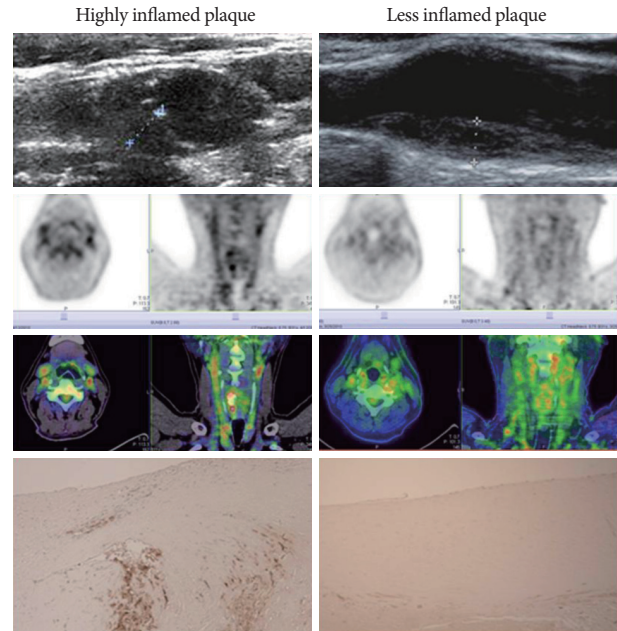
Fig. 5. Discrepancy between structure and inflammation during imaging. Both patients had a similar degree of stenotic plaques in their carotid arteries, but different positron emission tomography (PET) signals suggestive of greater inflammation in one patient (left column) and lower macrophage infiltration (lower panel) in the other patient (right column).

as a routine imaging technique. It is not specific to high-risk plaques and is heavily influenced by other factors such as fasting, stress, or medications. Additionally, the myocardium avidly takes up FDG, producing high background signals. PET imaging of coronary plaques has overcome the motion artifact and partial volume effects due to the small vessel size of coronary arteries. Radiation exposure is another concern for routinely adopting PET imaging.

Conclusion

In addition to traditional imaging targets such as macrophages, proteases, or adhesion molecules and promising targets including apoptosis and neo-angiogenesis, further advances in our understanding of atherosclerosis biology hold promise for identifying new targets. In the near future, molecular PET/CT using targeted ^{18}F or ^{68}Ga will be clinically introduced, mainly because of less concern for adverse contrast agent effects, compared with MRI, CT, or sonography. Fluorescence molecular imaging may be clinically introduced in the form of an intravascular NIRF-sensing catheter with the use of FDA-approved indocyanine green. The current rapid advances in translational molecular imaging research may fundamentally shift the diagnostic and therapeutic paradigm in the field of cardiovascular disease.

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