Molecular Imaging of Thrombus: Technology in Evolution

Running title: Lindner; Molecular imaging of thrombus

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For some time, scientists with expertise in medical imaging, cell biology, and chemical engineering have teamed together with the goal of producing targeted contrast agents for molecular imaging in cardiovascular disease. The goal of these efforts is to bring about techniques for non-invasively detecting and quantifying specific molecular processes that play a role in the pathophysiology of cardiovascular disease. Although there has been a tendency in the scientific literature to focus on how molecular imaging has been achieved, it is equally important to review why.

In the clinical setting, molecular imaging is likely to enhance and expand the diagnostic capabilities of current imaging applications. Nowhere has this been more successful than in cancer imaging where targeted contrast agents can detect otherwise non-apparent primary or metastatic disease or be used to select appropriate therapies based on tumor or tumor microvascular phenotype.¹ There are several areas of focus where molecular imaging could play an important role for early diagnosis and to guide patient management in cardiovascular disease. Imaging the cellular or molecular profile in atherosclerotic disease could yield important information on susceptibility for acute atherothrombotic complications.² In the future, it could also potentially be used to determine appropriateness for emerging therapies such as new drugs that interrupt the inflammatory response which will likely be expensive and have adverse effects.

Recent myocardial ischemia can be detected by molecular imaging of either a reduction in myocyte fatty acid utilization or post-ischemic inflammatory activation of the microvascular endothelium.³⁴ This approach could be used for rapid diagnosis in patients with chest pain of unclear origin and allow spatial assessment of the post-ischemic region even hours after resolution. Large-scale clinical trials with imaging of labeled branched-chain fatty acids have already been completed showing that the technique can accelerate the diagnosis of acute
coronary syndrome (ACS). Molecular imaging of sympathetic nerve activity could potentially be used to evaluate risk for life-threatening ventricular arrhythmia or adverse outcomes in patients with heart failure. In the research setting, molecular imaging is already being used in pre-clinical and clinical investigation as a toolkit to elucidate pathophysiology, or for rapid read-out of the efficacy of new therapies in animal models of disease or patients. Probably the best example of the latter is the use of fluorodeoxyglucose positron emission tomography (FDG-PET) in large vessels as an indirect marker of plaque inflammatory burden.

The likelihood that any given molecular imaging strategy will be translated into clinical practice relies largely on four considerations: (a) choosing the right molecular target; (b) being able to target the molecule effectively; (c) choosing the most appropriate imaging methodology (e.g. radionuclide imaging techniques, MRI, ultrasound, etc.); and (d) demonstrating incremental value to existing technology. It is in this context that one should examine the study by Wang et al. in this issue of Circulation, which describes ultrasound imaging of thrombus with microbubble contrast agents targeted to platelets.

Molecular imaging with ultrasound has generally involved conjugation of ligands to the surface of lipid-shelled microbubbles (mean diameter 2-3 μm) or other acoustically active nanoparticles at a site density of several hundred to several thousand per μm² surface area. Taking a lesson from nature, the ligands are usually projected away from the microbubble surface by polymeric molecular spacers to optimize steric conditions. For clot imaging, the use of an ultrasound-based approach is quite reasonable since microbubbles are pure intravascular tracers and have full access to the components of thrombus. Moreover, ultrasound molecular imaging has practical advantages in terms of speed (5-15 min protocols), portability, and cost. An important practical limitation is that imaging of thrombus in large coronary vessels is not
feasible from a non-invasive approach with currently existing ultrasound technology.

The notion that microbubbles can be targeted to thrombus is not new and was first reported almost 15 years ago. With respect to choosing the right target, thrombus formation or platelet-endothelial interactions have been imaged with microbubbles or other echogenic particulate compounds in animal models of disease include the platelet adhesion molecules $\alpha_{\text{IIb}}\beta_3$ integrin and GPIbα fibrin/fibrinogen, tissue factor and von Willebrand factor (VWF). While the description by Wang et al. of a contrast agent targeted to $\alpha_{\text{IIb}}\beta_3$ integrin to detect the platelet component in acute thrombus formation is not new, the strategy of using an antibody against a ligand-induced binding site (LIBS) on $\alpha_{\text{IIb}}\beta_3$ is novel and potentially important. Many of the previous attempts to target $\alpha_{\text{IIb}}\beta_3$ with microbubbles have used RGD-containing peptides or similar peptides as targeting ligands. While this provides a simple and low cost approach, the targeting efficacy of microbubbles bearing these peptides has been somewhat limited probably because of competitive inhibition from plasma components such as fibrinogen under high shear conditions, and their potential to bind to other integrins such as $\alpha_\text{IIb}\beta_3$ and $\alpha_\text{IIIb}\beta_1$. Antibodies against active site $\alpha_{\text{IIb}}\beta_3$ that bind irrespective of activation state such as abciximab have been used successfully to image human thrombus in vivo. The use of LIBS antibodies is potentially advantageous since they bind to sites exposed only upon integrin activation, thereby selectively attaching to activated platelets and reducing attachment to quiescent circulating platelets. Moreover, unlike non-activation-specific antibodies and RGD peptides, LIBS antibodies are less likely to trigger ligation-dependent platelet activation through “outside-in” signaling.

The study by Wang et al. demonstrates that microbubbles bearing LIBS antibodies attach to platelets or microthrombi, and enhance thrombi on ultrasound imaging of the murine carotid artery treated with FeCl. There are a few key steps that are needed in determining the impact of
this technologic advance. Most importantly, there now needs to be a direct comparison of microbubbles bearing LIBS antibodies to those targeted by either RGD peptides or non-activation-specific antibodies with regards to both microbubble binding efficiency and thrombus enhancement. Also, examining the influence of plasma on microbubble attachment to platelets in the flow chamber in this study would have been helpful for establishing the degree to which plasma proteins such as fibrinogen inhibit attachment of the LIBS-microbubbles under physiologic shear conditions.

It is worth noting that the peak signal enhancement that was achieved during in vivo imaging with LIBS-microbubbles was quite low (40% enhancement), substantially lower than that previously described for contrast ultrasound molecular imaging of the aorta in murine models of atherosclerotic disease where >10-fold enhancement has been achieved.\textsuperscript{14} It is unlikely that poor targeting efficiency was the primary reason. Instead low enhancement was likely a result of imaging methodology. High frequency (40 MHz), single pulse, fundamental (similar send and receive frequency) imaging may be ideal for defining thrombus in the murine carotid artery, however it is poorly suited to detecting microbubble signal. Instead low to intermediate frequencies with multi-pulse imaging algorithms that are specifically designed to detect microbubble non-linear signals are likely to increase signal relative to tissue signal for this agent like it has for most other microbubble agents.\textsuperscript{15}

As with any new molecular imaging technology that is developed and shown to be feasible, a critical question is whether targeted imaging of thrombus provides any unique or incremental value to what is already available to the researcher or clinician. The study by Wang et al. was designed to test feasibility rather than to show incremental value to non-contrast or non-targeted contrast imaging. In other words, we do not know whether contrast ultrasound with
LIBS-microbubbles improves the detection of small thrombi or provides greater accuracy for sizing thrombus over time. Although the authors state that thrombus imaging can be used to evaluate thrombolytic efficacy, one can certainly imagine other scenarios where molecular imaging of the platelet component of thrombus could have a positive impact. Targeted imaging may provide a unique opportunity to detect or study microvascular thrombus as a mechanism of no-flow in acute coronary syndromes (ACS). In large vessels, it could potentially detect microthrombi in stable patients or non-culprit vessels which have been correlated with heightened risk for plaque progression.\(^{16}\) In stroke or atrial fibrillation, it could be used to optimize anticoagulant therapy on a per patient basis. Platelet interaction with the intact endothelium which is partially mediated by dysregulation of VWF appears to contribute to the inflammatory status of atherosclerotic lesions, yet only recently has this process been imaged non-invasively.\(^{11}\) It should also be noted that applications may extend into the therapeutic realm where microbubbles have been shown to enhance coronary sonothrombolysis, the dissolution of clots using ultrasound energy.\(^{17}\)

There are many more potential cardiovascular or non-cardiovascular applications for ultrasound molecular imaging of thrombus that one can imagine if such technology were available. Borrowing some concepts from evolutionary biology, the introduction of new technology in medicine is gradual and requires “adaptive radiation”, the diversification of form to best suit a niche. Diversification is precisely what the study by Wang et al. provides; a new approach to thrombus imaging technology that may prove to be important for clinical application of molecular imaging in cardiovascular disease.

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