Chemokine receptors: Key for molecular imaging of inflammation in atherosclerosis

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Atherosclerosis, the leading cause of morbidity and mortality worldwide, is a progressive inflammatory disease characterized by the development of lipid-rich plaque lesions within arterial walls that extend into the vascular lumen. It is the underlying basis of cardiovascular diseases (CVD) including myocardial infarction, stroke, and peripheral arterial disease.[1] Despite major advances in risk factor modification, sophisticated anatomic and functional imaging tools, new therapeutics and state of the art revascularization techniques, by 2030 the prevalence of atherosclerotic heart disease and its complications are projected to increase by 12% causing significant financial burden.[2,3] In the clinical PET imaging of atherosclerosis, ^{18}F-fluorodeoxyglucose (^{18}F FDG) is the most often used radiotracer, although it provides little information about plaque status, vulnerability, likelihood of clinical event, or variation of inflammatory profile post treatment. Thus, investigative radiochemistry teams are putting their efforts toward developing molecular imaging agents that target biomarkers overexpressed during the initiation, progression, and potentially impending rupture of atherosclerotic lesions. Targets include vascular cell adhesion molecules, metalloproteinases, natriuretic peptide clearance receptors, and, most recently, chemokine receptors.[4–12] Some of these targeted radiotracers have been translated for human atherosclerosis imaging.[13,14]

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Wei and colleagues in their article in this issue of Journal of Nuclear Cardiology have developed a single photon emission computed tomography (SPECT) radiotracer that targets the chemokine receptor CCR5.[15] Chemokines are a group of small heparin-binding proteins. According to the position of *N*-terminal cysteine residue, the chemokines are organized into two major (CC and CXC) and two minor (C and CX3C) families. In review of basic animal studies and clinical research, over 20 chemokines are involved in plaque initiation, progression, destabilization, and rupture, playing critical roles in directing the movement of circulating leukocytes to the sites of inflammation or injury through their corresponding chemokine receptors.[16] During this longitudinal process, plaque composition is altered and the plaque is translated into a panel of secretory mediators and chemokine receptors. In humans, the upregulation of chemokine receptors in atherosclerotic lesions is well documented.[17] In a preclinical ApoE$^{-/-}$ mouse model of atherosclerosis, the progression of the aortic atherosclerotic lesions correlates well with increased expression of proinflammatory chemokine receptors. Of the myriad of chemokine receptors, CCR5 is of particular interest due to its known upregulation on monocytes/macrophages and T-lymphocytes. Deficiency of CCR5 in ApoE$^{-/-}$ mice has led to a significant reduction in plaque size and in the aortic proinflammatory cell population.[18] Moreover, with CCR5 antagonist treatment in ApoE$^{-/-}$ mice, plaque size has been shown to be reduced by 70% with macrophage infiltration attenuated by 50%.[19] All of these indicate a key role of CCR5 in plaque progression and its potential as an imaging biomarker for atherosclerosis.[17]

Wei and colleagues report development and assessment of a CCR5-targeted tracer using an ^{111}In radiolabeled DAPTA peptide (D-Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr-NH_2), a CCR5 antagonist. In their study, in vitro cell assays showed significantly higher uptake in U87-CD4-CCR5 cells compared to U87-MG cells, suggesting specific binding. Biodistribution in wild-type

C57BL/6 mice showed favorable pharmacokinetics and major clearance through renal system. Though high uptake in spleen was observed, it was mainly due to the accumulation of CCR5-expressing splenic monocytes. Autoradiography studies demonstrated much stronger lesion uptake of ^{111}In-DOTA-DAPTA in aortic arch of an ApoE$^{-/-}$ atherosclerotic mouse model than measured in C57BL/6 (normal) mice. Quantitative data analysis showed that the tracer accumulation in ApoE$^{-/-}$ mice fed on high-fat diet (HFD) for 4 months was 5 times higher than in age-matched C57BL/6 mice. Through competitive receptor blocking, the uptake in ApoE$^{-/-}$ mice was significantly decreased, confirming the targeting specificity. Interestingly, the authors also observed comparable tracer uptake in both ApoE$^{-/-}$ mice fed on standard chow and ApoE$^{-/-}$ mice fed a high-fat diet (HFD). ApoE$^{-/-}$ mice fed standard chow are known to develop atherosclerotic lesions at a slower rate. Moreover, the oil red O staining of lipid showed a profile similar to autoradiography, further supporting tracer binding to CCR5 receptor presence in atherosclerotic plaques. Taken together, these data provided a solid assessment of ^{111}In-DOTA-DAPTA in detecting atherosclerotic lesions in a classic mouse ApoE$^{-/-}$ model. The results suggest that ^{111}In-DOTA-DAPTA can specifically target CCR5 in atherosclerotic lesions and have potential for further evaluation.

There are, however, some limitations with the present study. Although a SPECT radiotracer has the potential to expand clinical atherosclerotic molecular imaging beyond the more limited PET scanner availability, the authors provide no ^{111}In-DOTA-DAPTA SPECT imaging of the atherosclerotic plaques of ApoE$^{-/-}$ mice. This is likely due to insufficient tracer uptake at plaque sites, a condition with potential to limit the clinical significance of the developed radiotracer. There are several approaches that may be taken to enhance the plaque uptake. The first option is to use a nanoparticle platform to which multiple copies of the DAPTA peptide could be conjugated. This would enhance the binding affinity of the radiotracer to CCR5 and afford improved uptake and visibility. Furthermore, CCR5 is a G-protein–coupled receptor, thus, upon the binding of the radiotracer to the CCR5 receptor, the receptor will be internalized and rapidly recycled back to the cell surface for successive binding. The extended blood circulation of nanoparticles would enable cumulative uptake of the radiotracer in plaques for improved target-to-background in contrast to peptide tracers, which typically have blood retention half-lives of no longer than a few minutes. However, the nanostructures need to be carefully designed to balance the targeting efficiency and pharmacokinetics.[9] A second option in preclinical development of the radiotracer is to use a large animal model such as rabbit, pig, or non-human primate. Indeed, it may be that the increased lesion size in these larger animals and, eventually in human subjects, with a greater number of CCR5 receptors would allow for more visibility in SPECT imaging.

Still, in preclinical assessment, these animal models need further histopathological characterization of CCR5 expression relative to plaque phenotype. Alternatively, the femoral artery injury mouse ApoE$^{-/-}$ model may be useful due to the acute nature of the model and elevated expression of the CCR5.[10]

Another limitation is that the authors did not perform a longitudinal study. A longitudinal study would characterize the tracer uptake in atherosclerotic lesions during progression of the atherosclerotic model. The expression of CCR5 in atherosclerotic plaques is dynamic and age-associated. CCR5 is reported to play central role in the development of late-stage plaque.[18] An extension of the current study may produce a more meaningful assessment of the potential of ^{111}In-DOTA-DAPTA to detect more vulnerable plaques, and would further elucidate clinical significance and potential impact of the radiotracer to predict outcome and direct therapy in human subjects.

A third limitation is the lack of histopathological characterization of the atherosclerotic lesions in the Apo E^{-}/$^{-}$ animals studied and the expression of CCR5 receptor on the plaque. Hematoxylin and eosin (H&E) staining, if performed, would have shown the histological features of the plaque, including severity and cellularity of the lesion, and immunohistochemical (IHC) staining would have shown CCR5 receptor expression levels and location (adventitia, media, intima) within the plaque. Performance of this additional assessment would further highlight the importance of CCR5 as a valuable biomarker for atherosclerosis imaging and therapy.

There is a growing body of work on developing targeted molecular imaging agents for atherosclerosis diagnosis due to its capability to visualize the expression and activity of particular molecules, cells, or functions that influence disease progression, outcome, and/or responsiveness to therapeutics. Because of the low and dynamic expression of biomarkers on advanced plaques, detection sensitivity, specificity, and translation capability of the imaging probes are valuable for the functional evaluation of plaque progression and activity.

In this study it is clear that ^{111}In-DOTA-DAPTA showed sensitivity and specificity in detecting atherosclerotic lesions. However, the lack of other key information limits the complete understanding of the pathophysiology underlying the radioactive signals.

Besides ^{111}In-DOTA-DAPTA reported herein for CCR5 imaging, other chemokine receptor targeted

imaging agents have also been reported including ^{68}Ga-Pentixafor for CXCR4[13] and ^{64}Cu-DOTA-ECL1i for CCR2.[20] Due to the critical role of chemokine receptors in mediating leukocytes trafficking to the site of inflammation during the initiation and progression of atherosclerosis, these non-invasive imaging agents either alone or in combination will be useful to help understand the proinflammatory cell variation of atherosclerosis dynamics. In particular, the broad applicability of a SPECT radiotracer such as ^{111}In-DOTA-DAPTA will assist in the translation to the clinical setting.

Disclosure

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