A novel PET tracer for targeted imaging of atherosclerosis

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Coronary atherosclerosis is the leading cause of myocardial ischaemia and infarction in the adult population of Western societies. Rapid and often sudden occlusion of the coronary arterial lumen by a ruptured and thrombosed atherosclerotic plaque is recognised as the most important mechanism in the pathogenesis of acute coronary syndromes. Atheromatous plaques at a high risk of rupture and thrombosis—also referred to as ''vulnerable'' plaques, exhibit characteristic features that distinguish them from low risk or ''stable plaques''. These include a large lipid-rich necrotic core, thin fibrous cap, extensive neovascularisation and plaque haemorrhage, adventitial inflammation, patchy calcification and expansive remodelling of the arterial wall.[1] So far, none of these histopathological features has been identified as the culprit ultimately responsible for plaque rupture and thrombosis but some features appear to play a more crucial role than others.

The recent prospective study of coronary atherosclerosis imaging by intravascular ultrasound in patients undergoing coronary angiography and percutaneous coronary intervention for an acute coronary syndrome or PROSPECT study identified the presence of thin-cap fibroatheromas as the strongest independent predictor of adverse cardiovascular events and hence of plaque instability.[2] Other ultrasound-defined features that were independently associated with a risk of adverse events included, in order of importance, a small luminal area ($\leq$4.0 mm^2) and a large plaque burden $\geq$70%. Their occurrence has incremental prognostic value with the highest risk of a major adverse outcome observed when all three were present. The prevalence of thinned-cap atheroma in the studied population was relatively high at 46.7%; however, only a small proportion of these plaques resulted in an adverse event. Moreover, the presence of all three features was associated with an event rate of only $\sim$18%. These observations were partly attributed to limitations inherent to tissue characterisation by intravascular ultrasound imaging. Therefore, it is possible that simultaneous assessment of other biological processes involved in the progression of atherosclerosis such as inflammation and neovascularisation may increase the power of structural changes for the prediction of plaque rupture and risk of cardiovascular events.

At present, coronary artery plaque characterisation in patients is best performed invasively with the use of ancillary modalities such as intracoronary ultrasound and optimal coherence tomography during coronary angiography but, as mentioned before, this approach has limitations including incomplete coverage of the coronary vascular tree and risk of potentially serious periprocedural complications.[2,3] This justifies the effort and resources currently spent in improving available methods while underscoring the importance of exploring new venues that may lead to a more comprehensive assessment of plaque composition and instability. In this regard, radionuclide imaging may provide a useful tool for the assessment of plaque vulnerability by allowing targeted visualisation of biomarkers of disease in a non-invasive manner. Among these, $\alpha v\beta 3$ integrin, a biomarker of neovascularisation, has become the molecular target for newly developed radiotracers.

Previous studies have already identified neovascularisation within the atherosclerotic plaque as an important feature of plaque progression that has the potential for increasing plaque vulnerability to rupture.[4,5] Varying degrees of neovascularisation within atherosclerotic lesions have been observed but ruptured plaques often exhibit higher degrees of new vessel

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formation compared with ''stable'' or fibrocalcified lesions.[3] Neovascularisation may contribute to plaque instability via rapid plaque expansion secondary to intraplaque haemorrhage, leakage of inflammatory precursors and extravasation of cells into the plaque core including erythrocytes and macrophages.[4,6] Results from in vitro studies of animal and human specimens have shown that new vessel formation occurs in the early stages of atherosclerosis in response to lipid accumulation within the arterial wall.[7,8] In later stages, formation of new vessels within the atheroma has been attributed to plaque growth and subsequent hypoxia.[7,9] This process involves complex molecular pathways including the release of growth factors and expression of adhesion molecules such as integrins. The latter are a key to cell surface-recognition mechanisms and play an important role in cellular migration and differentiation.

Integrins are a family of heterodimeric transmembrane glycoproteins that mediate the interaction of cells with other cells and/or with compounds of the extracellular matrix. $\alpha v\beta 3$ integrin, also known as vitronectin receptor, is highly expressed on the surface of activated endothelial cells during angiogenesis.[10] Early experimental observations on tumour-induced neovascularisation suggested that $\alpha v\beta 3$ expression was upregulated during neovessel development to facilitate cellular adhesion.[11] This is supported by studies showing that $\alpha v\beta 3$ antagonists can effectively prevent and/or reverse angiogenesis in models of tumour growth both in vitro and in vivo.[12,13] High expression of $\alpha v\beta 3$ integrin has also been found in atherosclerotic lesions, and this correlated with proliferating vascular endothelial cells within the lesions.[14] This integrin is, therefore, considered an important molecular marker of intraplaque angiogenesis.

In this issue of the Journal, Golestani et al. demonstrate the feasibility of [18F]-RGD-K5 imaging by high-resolution micro PET for the detection of $\alpha v\beta 3$ integrin expression in atherosclerotic plaque specimens of patients who underwent clinically indicated carotid endarterectomy. Specimens from 19 patients were incubated for one hour in a solution containing the radiotracer [18F]-RGD-K5. Accumulation of [18F]-RGD-K5 in the excised specimens correlated with the expression of $\alpha v\beta 3$ integrin on immunohistochemical staining. Moreover, there was a good correlation between [18F]-RGD-K5 uptake within the plaque and CD31 staining score, an indicator of endothelial cell density. A blocking experiment showed significant reduction in [18F]-RGD-K5 activity following incubation of a subset of specimens with an excessive amount of cold competing compound. This confirmed the specific binding of [18F]-RGD-K5 to $\alpha v\beta 3$ integrin.

A novel aspect of this work is the use of the radiolabelled peptide RGD-K5 to image integrin expression in atherosclerotic disease.[15] Like [18F]-Galacto-RGD, one of the first tracers of its type to be tested in experimental and clinical studies, [18F]-RGD-K5 belongs to a group of newly developed PET tracers that contain the RGD sequence.[16] This short aminoacid sequence (Arg-Gly-Asp) acts as a binding site for a subset of integrins including $\alpha v\beta 3$. A number of RGD-containing peptides have been developed for targeted PET imaging of integrin expression in tumours [17-19] but only a few are being tested for imaging integrin in atherosclerosis. [18F]-RGD-K5 is a selective and high-affinity tracer for $\alpha v\beta 3$ integrin with Kd 7.9 nM.[20] Moreover, it exhibits a favourable biodistribution and safety profile in healthy volunteers with rapid clearance from blood ($\sim$12 minutes) and primary excretion through the kidneys. The highest [18F]-RGD-K5 activity was found in the renal system, liver and guts while a near-to-background activity was observed in the heart following injection.[20]

The findings of Golestani et al. confirm earlier observations from experimental models and clinical studies of $\alpha v\beta 3$ expression in malignant tumours and its relation to tumour growth and neovascularisation.[17,18,21] Moreover, they support the hypothesis that imaging of $\alpha v\beta 3$ targeting RGD-peptides may enhance plaque characterisation both in vitro and in vivo by allowing the detection of intraplaque angiogenesis and potentially providing a quantitative estimate of neovascularisation burden. Indeed, Golestani et al. demonstrated a good correlation between [18F]-RGD-K5 uptake and intraplaque neovessel density.

It was not specifically addressed in this study whether tracer uptake in the specimens could also be attributed to macrophage infiltration. $\alpha v\beta 3$ integrin is expressed on the surface of macrophages, which play a crucial role in the pathogenesis of atherosclerosis by mediating an inflammatory response that may lead to plaque expansion and increase vulnerability to rupture.[22,23] A large macrophage infiltrate would be expected in high-grade atherosclerotic lesions like the ones analysed in the current study. Indeed, Beer et al. recently documented the expression of $\alpha v\beta 3$ integrin in macrophage infiltrates of plaque specimens obtained from patients with high-grade carotid artery stenosis.[24] Therefore, it is possible that in the current study a proportion of measured activity within the plaque might reflect tracer binding to $\alpha v\beta 3$ integrin molecules expressed on the surface of macrophages. In the same study of Beer et al. immunohistochemical CD68 staining analysis showed macrophage infiltration in the plaques to a variable extent, and there was only a weak correlation between CD68 staining intensity and plaque [18F]-Galacto-RGD uptake.[24] It is already known that macrophages are abundant in advanced and rupture-

prone lesions, supporting their pivotal role in disease progression, but their density varies within the atherosclerotic plaques.[1,25] Moreover, expression of αvβ3 integrin in coronary atheromas has been colocalised with other cell types including smooth muscle cells outside regions of neovascularisation.[14] All these observations suggest that αvβ3 integrin-targeted imaging may not be specific for intraplaque neovessels and interpretation must consider the potential contribution of other sources of αvβ3 integrin expression to imaging findings.

It is fair to say that when using [18F]-RGD-K5 PET imaging alone for the purpose of plaque characterisation in vivo, it may not be possible to attribute the resulting tracer signal to a specific biological process. However, this should not be perceived as a shortcoming; as the authors pointed out, [18F]-RGD-K5 imaging could prove useful for the simultaneous assessment of neovascularisation and inflammation; it is even possible to speculate that, by providing a combined estimate of neovascularisation and inflammation burden, this approach may be more accurate than the assessment of individual pathological processes at identifying plaque vulnerability and predicting risk of rupture.

Another important contribution of the study of Golestani et al. was to provide further evidence that high-resolution micro PET is a reliable method for the detection and characterisation of tracer activity in specimens of atherosclerosis that can be readily compared with results from histochemical analysis of molecular targets. In this study, [18F]-RGD-K5 uptake was observed in all specimens but its distribution was heterogeneous within and between the plaques. This has been a consistent finding in previous studies; a variable binding and uptake of radiolabelled RGD compounds has been described in models of malignancy and atherosclerosis.[24,26-28] There was no histopathological correlate in the current study to examine the relation between αvβ3 integrin expression by [18F]-RGD-K5 uptake and plaque composition. The study of Hoshiga et al. has already shown that, in human coronary atherosclerosis, the expression of αvβ3 integrin is variable within the plaques.[14] A high expression of this integrin was identified in areas of increased cellularity but not all cell clusters were αvβ3 positive. The most prominent and uniform expression of αvβ3, however, was observed in intraplaque microvessels as well as in the adventitial vasa vasorum supporting the strong relation between αvβ3 integrin expression and angiogenesis. The variable degrees of αvβ3 integrin expression observed among atherosclerotic plaques may reflect different stages of the atherosclerotic process across and possibly within the same patient specimens. This would support the concept of atherosclerosis as a complex and dynamic process whereby plaques deemed clinically significant may actually differ in their cellular and molecular makeup.

The current study also provides useful data for future clinical trials. A high hot spot to total tracer activity ratio within the specimens was observed and this is consistent with findings from studies demonstrating good image contrast when using [18F]-RGD-containing compounds for imaging atherosclerosis in vivo.[24,27] Based on this pre-clinical observation, [18F]-RGD-K5 PET imaging may prove feasible for characterisation of atherosclerotic plaques in patients. It is important to bear in mind that these observations are linked to advanced atherosclerosis, and therefore the role of [18F]-RGD-K5 PET imaging in early stages of the disease remains to be established.

As demonstrated by the study of Golestani et al. imaging of carotid atherosclerosis provides a valuable opportunity for validation of novel diagnostic procedures and frequently represents the first step for in vivo imaging of atherosclerosis in humans. Further work is needed to assess the feasibility and potential application of radiolabelled RGD-containing PET tracers for the clinical evaluation of coronary atherosclerosis. One important limiting factor is the relatively lower spatial resolution of PET systems compared with other imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI). Further developments in hybrid imaging promise to enhance the scope of molecular imaging by providing the anatomic reference needed to enable accurate localisation of tracer signal. In this regard, recent studies have demonstrated the feasibility and potential application of [18F]-FDG and [18F]NaF PET/CTA imaging for the detection of actively inflamed atherosclerotic plaques within the coronary tree.[29,30] Currently, alternative methods to radionuclide techniques for targeted imaging of atherosclerosis are under investigation including MRI of gadolinium-based nanoparticles.[31] In an animal model of atherosclerosis, Winter et al. demonstrated specific accumulation of αvβ3 integrin-targeted paramagnetic nanoparticles in areas of neovessel proliferation within atherosclerotic aortic walls. The results of this study would support the potential application of αvβ3 integrin-targeted MRI for in vivo detection of intraplaque angiogenesis. There are other potential applications for targeted molecular imaging of atherosclerosis including the use of molecular targeting compounds to direct and monitor the delivery of drugs (e.g., integrin inhibitors) that may halt the progression of atherosclerosis.[10]

In conclusion, the work by Golestani et al. lends further support to non-invasive imaging of biological markers of atherosclerosis. This study demonstrates that visualisation of αvβ3 expression in specimens of

atherosclerotic disease is feasible using [18F]-RGD-K5 PET imaging, and supports previously published data on the potential applications of these newly developed tracers for the detection of angiogenesis and inflammation in atheromatous plaques. These results are encouraging and further research would be needed to determine not only the feasibility of [18F]-RGD-K5 PET imaging in the clinical setting but also its contribution to the assessment of plaque progression and vulnerability in vivo.

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