Molecular imaging of inflammation for detection of vulnerable atheromatous plaques

Juan Carlos Kaski*

Cardiovascular Sciences Research Centre, St George’s, University of London, Cranmer Terrace, London W4 3LW, UK

This paper was guest edited by Prof. Filippo Crea, Universita Catolica del Santo Cuore, Istituto di Cardiologia, Roma, 00168, Italy

Inflammation plays a major pathogenic role in the development of atheromatous plaques and the progression of atherosclerosis. Disruption of the fibrous cap of atheromatous lesions has been shown to lead to acute cardiovascular events. Atheromatous plaque disruption is the mechanism most commonly associated with the occurrence of acute coronary syndrome, i.e. unstable angina and acute myocardial infarction. Plaque disruption is directly linked to inflammation, particularly to macrophage activation, which results in the release of proinflammatory cytokines and metalloproteases that contribute to the destruction of the fibrous cap of the atheroma thus weakening its structure and leading to the development of fissures that allow a direct contact between the prothrombotic atheromatous core and the circulating blood in the affected vessel. This process can result in the development of acute thrombosis and serious clinical events. The early identification—in a given individual—of plaques prone to disruption (‘vulnerable plaques’) is desirable, as this may allow the implementation of preventative strategies and, possibly, effective therapeutic intervention. Vulnerable plaques often show large necrotic core volumes, positive vascular remodelling, and attenuation of fibrous plaque caps (Figure 1).

Imaging techniques for detection of vulnerable plaques and individuals at increased risk

Angiographic and ultrasound plaque morphology and circulating biomarkers have been proposed as markers of disease progression and plaque rupture. However, the yield of this approach has been less rewarding than initially anticipated, particularly in relation to its use in the clinical setting. The search for effective markers of plaque vulnerability and the metabolic changes that take place within atherosclerotic plaques prone to disruption has therefore continued unabated. Imaging techniques such as positron emission tomography (PET) and contrast ultrasonography have been proposed as suitable tools for this purpose, and the possibility in recent years to use complementary multimodal imaging methods, such as computed tomography (CT), magnetic resonance imaging (MRI), and single photon emission computed tomography (SPECT), represents a welcome addition to our current armamentarium for the assessment of atherosclerotic plaque biology. PET, in particular, is of major interest as this technique allows the non-invasive assessment and quantification of metabolic processes that take place in the tissue itself. The most commonly used imaging agent for this purpose has been [18F]fluorine-labelled 2-deoxy-D-glucose (FDG). Its use has been most probably encouraged by the large body of evidence generated with this agent in cancer imaging. Studies have shown that PET can detect increased FDG uptake in atheromatous plaques, and a correlation between FDG uptake and macrophage content has been documented in atherosclerotic lesions. Evidence that molecular imaging is suited for the identification of monocyte–macrophage infiltration in the setting of acute vascular events in humans is convincing. However, the association reported in the literature, between macrophage content in atheroma and FDG uptake, is not a universal finding. Experimental studies in animals showed a poor correlation between [14C]2DG imaging signals and macrophage concentrations in aortas from ApoE-deficient mice. Furthermore, it has been shown that the [3H]2DG signal from rabbit plaques on autoradiography originates not only from macrophage-rich areas, but also from areas with abundant numbers of smooth muscle cells (SMCs).

* Corresponding author. Tel: +44 208 725 3963, Fax: +44 208 725 3328, Email: jkaski@sgul.ac.uk

The opinions expressed in this article are not necessarily those of the Editors of the European Heart Journal or of the European Society of Cardiology.

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2012. For permissions please email: journals.permissions@oup.com
Interest in this regard, as it showed that hypoxia, but not necessarily proinflammatory cytokines, stimulated glucose uptake in human macrophages and foam cells. Immunohistochemical assessment of human plaques in the study revealed abundant expression of proteins regulating glucose utilization, predominantly in macrophage-rich regions of the atheromatous plaque, regions previously proved hypoxic. Of importance, vascular SMCs and endothelial cells showed markedly increased rates of glucose uptake when exposed to proinflammatory cytokines. Thus these findings seem to indicate that glucose uptake and, most probably, FDG uptake signals in atheroma may reflect hypoxia-stimulated macrophages and not just purely inflammatory mechanisms. Inflammatory cytokines activated SMCs, and this also may contribute to the FDG signal detected in experimental and clinical studies.

**Imaging of inflammation with $^{[11]C}$PK11195**

Gaemperli *et al*.\(^{15}\) have used the imaging agent $^{[11]C}$PK11195, a selective ligand of the translocator protein (TSPO), highly expressed in activated macrophages, to determine whether intraplaque inflammation could be reliably measured using combined PET and computed tomography angiography (PET/CTA) imaging. To this end, they recruited symptomatic and asymptomatic patients with carotid artery stenoses. In those patients who underwent carotid endarterectomy, ultrathin contiguous sections were processed for TSPO and CD68, using immunohistochemical staining, $^{[3]H}$PK11195 autoradiography, and confocal fluorescence microscopy. On immunohistochemistry and confocal fluorescence microscopy, CD68 and the peripheral benzodiazepine receptor (PBR) were found to co-localize with $^{[3]H}$PK11195 uptake at autoradiography. $^{[11]C}$PK11195 uptake into carotid plaques was measured using target-to-background ratios (TBRs)—a way of assessing the presence of the imaging agent $^{[11]C}$PK11195, a selective ligand of TSPO—and one of the main findings in the study was a significant correlation between the $^{[11]C}$PK11195 TBR and autoradiographic percentage specific binding ($r = 0.77$, $P = 0.025$), thus confirming the feasibility of assessing plaque and vascular inflammation with combined PET/CTA. The study also showed that patients with a recent cerebral ischaemic event had ipsilateral plaques with lower CTA attenuation and increased $^{[11]C}$PK11195 uptake.
Molecular imaging

$[1^{11}C]PK11195$ had been used successfully for the in vivo imaging of brain and vascular tissue inflammation. Studies using $[1^{11}C]PK11195$ PET in neurodegenerative, inflammatory, and neoplastic brain disorders showed that $[1^{11}C]PK11195$ PET was useful for the identification of vascular inflammation and assessment of treatment. This type of in vivo imaging can document the expression of PBR, directly linked to microglial activation, and has been considered to represent a hallmark of neuroinflammation. In vascular tissue, Pugliese et al. showed, in symptomatic patients with systemic inflammatory disorders and active vasculitis, that PET/CT with $[1^{11}C]PK11195$ identified the inflammatory process in the arterial wall. Of interest, there was a good correlation between the uptake of $[1^{11}C]PK11195$ and the healthy vascular wall. Although the uptake of $[1^{11}C]PK11195$ was higher in atherosclerotic plaques containing a large number of inflammatory cells compared with non-inflamed plaques, the tracer uptake by other structures of the arterial wall was also prominent. Several new radioligands for the PBR are currently being evaluated that could successfully challenge $[1^{11}C]PK11195$'s pre-eminence as a marker of plaque inflammation. Moreover, studies have proposed the use of nanoparticle PET-CT imaging of macrophages in inflammatory atherosclerosis as a more suitable biomarker. These studies have shown the capability of a novel tridmodality nanoparticle to detect macrophages directly in atherosclerotic plaques. Some of the advantages of this approach include: improved sensitivity, a direct correlation of the PET signal with established biomarkers of macrophage activation (i.e. CD68), the ability to quantify the PET signal, and the possibility of performing whole-body vascular surveys, and to spatially localize and follow the nanoparticle by microscopy. Conceivably, more comprehensive information regarding the complex mechanisms associated with plaque vulnerability and inflammation could be obtained using a multimarker approach. Recent work with a combined double tracer, $[1^{11}C]PK11195$ and $[1^{18}F]FDG$-PET, has shown in a brain ischemia rat model, that after permanent focal ischemia, neuroinflammation develops in the normoperfused peri-infarct zone and correlates with increased energy demand that in turn results in tissue damage of areas adjacent to the infarct. It has been reported that information obtained with these two imaging modalities, FDG and PET/CT, is complementary and can enhance the diagnostic performance of the hybrid technique to detect potentially vulnerable plaques.

Lessons from the study and future directions

Despite some obvious limitations, the study by Gaemperli et al. represents a step forward in the understanding of plaque biology and, like other studies in the field previously, confirms the feasibility of detecting plaque inflammation non-invasively. Unfortunately, the study has little power to allow extrapolation of its findings to the clinical setting and/or enlighten clinicians on the role of this technique in secondary prevention. However, this ‘proof of principle’ study is important, as it shows the potential of this non-invasive multimodal approach for the integral assessment of vascular inflammation and plaque vulnerability in patients. Unfortunately, the study did not compare the diagnostic and prognostic roles of imaging vs. systemic markers of inflammation, and this should be an avenue for future research. Findings in this study are of interest and should stimulate further investigation into the true role of vascular inflammation imaging in the clinical setting. Large, well-designed studies are warranted, in real-life patients, that comprehensively address this issue. Clinicians should be eager to embrace a technique that accurately allows the early identification of vulnerable plaques and ‘vulnerable’ patients, as such a tool is likely to improve risk stratification and help in implementing successful preventative and therapeutic strategies.

Conflict of interest: none declared.

References

2. Moreno PR, Falk E, Palacios IF, Newell JB, Fuster V, Fallon JT. Macrophage infiltra-
4. Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical con-
7. Falco EJ, Shekine Y, Rucha VZ, Christen T, Szurzok E, Sukhova GK, Di Carli MF, Libby P. Hypoxia but not inflammation augments glucose uptake in human-


