Vascular Pharmacology 58 (2013) 31–38

Contents lists available at SciVerse ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Review

Targeted molecular imaging of vascular inflammation in cardiovascular disease using nano- and micro-sized agents

Martina A. McAteer *, Robin P. Choudhury

Division of Cardiovascular Medicine, Radcliffe Department of Medicine, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DU, United Kingdom

ARTICLE INFO

Article history:
Received 18 October 2012
Accepted 19 October 2012

Keywords:
Molecular imaging
Magnetic resonance imaging
Adhesion molecules
Atherosclerosis
Endothelial activation
Vascular inflammation

ABSTRACT

Molecular imaging is emerging as a key experimental tool for the identification of inflammatory cellular and molecular processes involved in the development of cardiovascular disease. This review summarises current molecular imaging approaches for the detection of vascular inflammation using a range of nano- and micro-sized contrast agents. We highlight strategies for detection of cell adhesion molecules, which are key regulators of endothelial activation and leukocyte recruitment in atherogenesis and ischaemia–reperfusion injury. In particular, we address the properties of targeted microparticles of iron oxide (MPIO) for MRI detection of endothelial cell-specific activation of adhesion molecules in experimental models of atherosclerosis, acute vascular inflammation and ischaemia–reperfusion injury, which are otherwise undetectable by conventional imaging modalities. The ability of targeted MPIO to detect endothelial activation could enable early subclinical disease detection and development of novel therapeutic strategies. We discuss opportunities for further development and potential translation of targeted MPIO for clinical imaging of cardiovascular disease.

Contents

1. Introduction

Atherosclerosis is a chronic inflammatory disease of medium to large arteries, characterised by endothelial activation and accumulation of inflammatory cells within the vessel wall. Conventional clinical imaging of cardiovascular disease by X-ray angiography, can detect the severity of coronary luminal stenosis and carotid intima-media thickening, and can guide interventions such as surgical coronary revascularization and primary percutaneous coronary intervention (PPCI). However, autopsy studies show that most fatal myocardial infarctions (MI) are due to atherosclerotic lesions that do not cause flow-limiting stenosis (Virmani et al., 2006) and therefore would not be detectable using standard X-ray angiography. Techniques such as intravascular ultrasonography (IVUS) (Hartmann et al., 2011) and optical coherence tomography (OCT) (Rieber et al., 2006) can enable characterisation of atherosclerotic plaque composition but cannot report specifically on the inflammatory processes that drive atherosclerotic lesion development. Therefore,

* Corresponding author. Tel.: +44 1865 234647; fax: +44 1865 234681.
E-mail address: martina@well.ox.ac.uk (M.A. McAteer).

1537-1891/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.vph.2012.10.005

imaging approaches are urgently needed for improved detection of inflammatory lesions and for assessment of novel targeted therapeutics in patients at future risk of clinical events, such as MI or ischemic stroke.

Molecular imaging is emerging as a novel approach to identify inflammatory processes in atherosclerosis at the molecular and cellular level. Key steps in the inflammatory cascade include vascular endothelial dysfunction and activation of cell adhesion molecules, monocyte recruitment and differentiation into macrophages, proteolysis and extracellular matrix degradation, apoptosis and angiogenesis (Choudhury et al., 2004). Endothelial activation is a key event in early atherogenesis, characterised by the up-regulation of adhesion molecules, vascular cell adhesion molecule-1, (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), P- and E-selectin, promoting monocyte recruitment to the vascular wall and subsequent lesion development. Arterial regions exposed to low shear stress and disturbed flow, such as the inner curvature of the aortic arch and bifurcations, show increased endothelial activation and susceptibility to developing atherosclerotic lesions (Davies et al., 1993; Nakashima et al., 1998; Ramos et al., 1999). Initial monocyte rolling along activated endothelium is mediated by P-selectin and its interaction with integrin P-selectin glycoprotein ligand-1 (PSGL-1) expressed on monocytes, while firm adhesion of monocytes is mediated by VCAM-1 (CD106) and engagement of the integrin very late antigen-4, VLA-4 (also known as $\alpha_4\beta_1$ integrin) expressed on monocytes (Dansky et al., 2001). VCAM-1 is a promising marker for molecular imaging of vascular inflammation in atherosclerosis, since it is not constitutively expressed in normal vessels but is rapidly up-regulated on vascular endothelial cells in both early and advanced lesions (Cybulsky et al., 2001; Davies et al., 1993) and is readily accessible to blood-borne, targeted contrast agents. VCAM-1 is also up-regulated by macrophages and smooth muscle cells in atherosclerotic plaques (Li et al., 1993; Libby and Li, 1993).

Approaches to in vivo molecular imaging of vascular adhesion molecules in cardiovascular disease have included monoclonal antibody and peptide ligands covalently linked to (i) magnetofluorescent nanoparticles for magnetic resonance imaging (MRI) and intravital microscopy/fluorescent molecular tomography (FMT) (Nahrendorf et al., 2006), (ii) microbubbles for contrast enhanced ultrasound (CEU) (Barreiro et al., 2009; Kaufmann et al., 2010; Kaufmann et al., 2007b; Lindner et al., 2001; Villanueva et al., 2007) or (iii) radiolabels (fluorine-18 (^{18}F) or technetium-99m (^{99m}Tc)) for hybrid positron emission tomography (PET)–computed tomography (CT) (Nahrendorf et al., 2009) or single positron emission computed tomography (SPECT) imaging, respectively (Broisat et al., 2012). We have adopted a micro-sized approach for in vivo molecular MRI of vascular endothelial inflammatory responses using microparticles of iron oxide (MPIO) (McAteer et al., 2010; McAteer and Choudhury, 2009; McAteer et al., 2012). Due to their confinement to the intravascular compartment, we have designed MPIO that specifically target endothelial cell activation and leukocyte adhesion, the key regulatory events in atherogenesis. In this review, we discuss nano- and micro-sized contrast agent approaches for cellular and molecular imaging of vascular inflammation in cardiovascular disease by nuclear imaging, MRI and CEU imaging. In particular, we highlight the properties of ligand-targeted MPIO for molecular MRI of endothelial inflammation in experimental models of acute vascular inflammation (McAteer et al., 2007; Serres et al., 2011, 2012), atherosclerosis (McAteer et al., 2008, 2010, 2012) and ischaemia–reperfusion injury (Akhtar et al., 2010; Hoyte et al., 2010). For atherosclerosis imaging, we discuss the ability of leukocyte-mimetic MPIO to discriminate endothelial cell-specific activation during early atherosclerotic lesion development using in vivo MRI and the cellular specificity of MPIO binding to atherosclerosis-susceptible sites (McAteer et al., 2012). The limitations and translational potential of ligand-targeted MPIO for clinical imaging of cardiovascular disease are also discussed.

2. Molecular imaging of vascular inflammation in cardiovascular disease

2.1. Nuclear imaging

Nuclear imaging techniques such as PET or SPECT allow quantitative measurements of atherosclerotic plaques with high sensitivity. PET has higher temporal resolution and sensitivity relative to SPECT, and can detect radiotracers such as ^{18}F in the nanomolar to picomolar (10^{-9} M to 10^{-12} M) range. For in vivo vascular imaging using PET, radiotracer ^{18}F-fluorodeoxyglucose (FDG) has been shown to accumulate in macrophage-rich atherosclerotic plaques (Rudd et al., 2009; Tahara et al., 2006). However, recent in vitro studies suggest that FDG accumulation may reflect hypoxia-stimulated macrophages rather than inflammation per se (Folco et al., 2011). For molecular imaging of vascular inflammation, Nahrendorf et al., 2009 have developed a VCAM-1 targeted radiotracer, termed ^{18}F-4V, which is internalised by endothelial cells and can detect VCAM-1 expression in murine atherosclerotic plaques by in vivo hybrid PET–CT (Nahrendorf et al., 2009). Reduced lesional uptake of ^{18}F-4V was detected in mice treated with atorvastatin, which corresponded to reduced aortic VCAM-1 mRNA expression. The fast blood clearance kinetics of ^{18}F-4V enabled imaging to begin 1 h after administration, with minimal background signal.

Recently, nanobodies recognising both human and mouse VCAM-1 have been investigated as potential targeting ligands for SPECT (Broisat et al., 2012). Nanobodies are single-domain antibody fragments that occur naturally in sharks and camelids. Using non-invasive SPECT–CT imaging, ^{99m}Tc-radiolabelled VCAM-1 nanobodies enabled in vivo detection of VCAM-1 expression in aortic arch atherosclerosis in apolipoprotein E deficient (apoE$^{-/-}$) mice. The fast blood clearance of nanobodies enabled imaging to commence 2 h after nanobody administration. Fucoidan, which is a naturally occurring polysaccharide mimetic of sLe^x, derived from brown seaweed has also been investigated as a targeting ligand for P-selectin. Using a rat model of ischaemia–reperfusion injury, ^{99m}Tc-labelled fucoidan was shown to detect endothelial activation in the ischemic myocardium 2 h after reperfusion (Rouzet et al., 2011). However, SPECT and PET modalities remain limited by poor spatial resolution, exposure to ionising radiation and expense.

2.2. MRI

MRI is a leading imaging modality for assessment of cardiovascular disease as it provides high spatial and temporal resolution images of the vascular wall, with excellent soft tissue contrast, without the use of ionising radiation. The inherent low sensitivity of MRI compared to PET and SPECT can be overcome by the use of paramagnetic gadolinium (Gd) or super-paramagnetic iron oxide nanoparticle contrast agents. Targeted delivery of these agents has been a focus of intense research and can be accomplished by covalent conjugation of ligands, such as monoclonal antibody or peptide ligands to the nanoparticle surface. Below we highlight the advantages and limitations of Gd and iron oxide based agents for cellular and molecular MRI of vascular inflammation in cardiovascular disease.

2.3. Gadolinium-based agents

Gd-based agents such as liposomes (Sipkins et al., 1998), perfluorocarbon lipid emulsions (Yu et al., 2000), micelles (Amirbekian et al., 2007; Lipinski et al., 2006) and lipoproteins (Frias et al., 2004; Glickson et al., 2008) carrying substantial payloads of amphipathic Gd chelates embedded in their outer membrane have demonstrated potential for molecular MRI of macrophages in atherosclerotic lesions. Gd-based nanoparticles conjugated to sugar ligand sialyl Lewis^x (sLe^x), have been shown to detect E-selectin up-regulation during early cerebral endothelial activation by in vivo MRI (Sibson et al.,