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Platelets, Haemostasis and Inflammation

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Preface

Over the last two decades, inflammation has emerged as the key underlying pathology of a variety of diseases including but not limited to rheumatoid disorders, cancer, and cardiovascular disease. Inflammation within the vessel wall clearly promotes the nascence of atherosclerotic lesions and consequently the rise of clinical complications such as myocardial infarction and stroke. A plethora of basic and clinical evidence clearly links this inflammatory process with disease burden. We appreciate today that atherosclerotic lesions containing a multitude of inflammatory cells tend to be much weaker in composition and consistence, rendering them more prone to rupture and subsequent clinical sequelae. While the role of classic inflammatory cells and immunologic cell types has been extensively characterized throughout the last decade, it only recently became evident that nontraditional inflammatory cell types such as the platelet take a center stage in initiation, promotion, and ultimately complication of vascular inflammation. This book focuses on the platelet as a versatile cell type unraveling its role as a mediator between hemostasis and inflammation. Finally, we propose several platelet-targeting and alternate anti-inflammatory therapies as novel and promising therapeutic approaches to ultimately combat the high residual risk of cardiovascular disease in our world.

We thank our internationally renowned faculty for their outstanding contribution and wish you, our readers, joy and enlightenment with our book.

Freiburg, Germany — Andreas Zirlik
Freiburg, Germany — Christoph Bode
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Constantin von zur Mühlen and Robin P. Choudhury

Abstract

Platelets and inflammation play a pivotal role in a wide range of cardiac pathophysiologies, such as coronary vessel atherosclerosis, ischemia/reperfusion injury, or myocarditis. Imaging of early stages of these diseases would be helpful. Molecular imaging is a promising approach for characterizing biological processes and especially atherosclerosis, which presents numerous mechanistically important targets. Inflammation and thrombus formation as key events are reflected by a wide range of potential targets, e.g., inflammatory adhesion molecules, inflammatory cells and proteases, or fibrin and platelets. Molecular imaging of these processes is possible by applying single imaging techniques, such as MRI, or the combination of different imaging modalities, such as PET and CT. In this chapter, we describe current concepts, challenges, and the future potential of molecular imaging in the context of platelets and inflammation involved in atherosclerosis.

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1.1 Introduction

Platelets and inflammation play a pivotal role in a wide range of cardiac pathophysiologies, such as coronary vessel atherosclerosis, ischemia/reperfusion injury, or myocarditis [1–3]. Imaging of early stages of these diseases would be helpful to manage the patient, and imaging of established disease could help to guide or optimize treatment. Various imaging techniques are available, either already clinically established or at an experimental level. In this chapter, different imaging approaches to target platelets and/or inflammation will be described, with a focus on vascular inflammation and thrombosis in atherosclerosis. We describe techniques applied in animal studies, but also in humans, and the challenging path from "bench to bedside."

1.2 Understanding Coronary Atherosclerosis: Still a Long Way to Go

Years ago the idea of atherosclerosis development was very simple. Depositions of fatty tissue, so-called "fatty streaks," already develop during early childhood [4]. Over time and with the exposure toward certain risk factors, they progress toward atherosclerotic plaques, progressively resulting in luminal narrowing and symptoms in the patient. At some point, plaque rupture occurs, and a rapid superimposed thrombosis results in immediate vessel occlusion and therefore myocardial infarction or stroke [1, 5]. However, we have learned that it is not such a linear progression of disease and that smaller and nonobstructive plaques can rupture abruptly and cause vascular occlusion [6]. Such plaques are often missed by conventional imaging techniques available in routine clinical practice. A coronary angiogram, which is routinely performed in patients with symptoms suggestive of coronary artery disease (CAD), only shows the luminal filling with contrast agent but cannot characterize the occult vascular inflammation of the vascular wall that does not result in significant luminal narrowing. In 2011, the "PROSPECT" study was published, which tried to characterize nonocclusive lesion in patients with an acute coronary syndrome (ACS) [7]. Patients with an ACS and therefore subtotal/total occlusion of a coronary vessel were treated by percutaneous coronary intervention (PCI) and stent placement at the so-called "culprit" lesion. Nonocclusive, non-culprit lesions were characterized by gray-scale and radiofrequency intravascular ultrasonographic imaging (IVUS) after PCI, and median follow-up period was 3.4 years. 20.4% of patients came back with new major adverse cardiac events

(death from cardiac causes, cardiac arrest, myocardial infarction, or rehospitalization due to unstable or progressive angina). However, only 12.9% of these new events were related to the initially treated culprit lesion; the other 11.6% were related to non-culprit lesions, which were angiographically mild at baseline. These potentially "vulnerable" non-culprit lesions were characterized by a plaque burden of 70% or greater, or a minimal luminal area of 4.0 mm^2 or less, or were classified as thin-cap fibroatheromas (TCFA) by virtual histology in IVUS. However, also the combination of these different nonocclusive plaque characteristics did not result in a reliable prediction of MACE: when combining TCFA, plaque burden, and MLA, only 18.2% of patients with MACE had these characteristics present in the initial coronary angiogram.

Although these data might provide us with some prognostic information on IVUS-VH, we need other techniques to image more selectively plaque components and characteristics that might allow us to more precisely predict the fate of a coronary plaque. Especially targeting cells involved in certain stages of vascular inflammation or thrombosis by molecular imaging is an interesting and promising strategy.

1.3 Molecular Imaging: Definition, Goals, and Imaging Techniques

Molecular imaging can be defined as visualization, characterization, and noninvasive measurement of biological processes at the molecular and cellular levels in humans and other living systems [8]. This could help to accelerate and refine diagnosis, provide insights that reveal disease diversity, and monitor the effects of therapies. Molecular imaging contrast agents usually consist of two components: an antibody or peptide-mimetic targeting a certain cell or cellular receptor, conjugated toward a signal-giving carrier element. It now depends on which imaging will be used: for magnetic resonance imaging (MRI), paramagnetic chalets such as gadolinium (Gd) or superparamagnetic iron oxide particles (SPIOs) are attractive. While Gd causes a positive contrast in T1-weighted MRI, SPIO or in general iron oxide-based contrast agents result in a negative contrast due to susceptibility artifact in T2*-weighted MRI sequences [9, 10]. SPIOs are available in different sizes, e.g., as ultrasmall SPIOs (USPIOs) or microparticles of iron oxide (MPIOs). Depending upon the size and formulation, particles can be loaded with different quantities of iron and therefore have variable effects on susceptibility. These artifacts appear as black signal extinctions in T2*-weighted MRI, and 1 μm-sized MPIOs can cause signal effect extending their effective diameter on the image by a factor of 50.

When choosing a strategy of PET or SPECT for molecular imaging, radionuclides are usually conjugated with the targeting antibody or peptide mimetic. For ultrasound molecular imaging, air-filled microbubbles can be used to cause imaging artifacts.

The selection of the optimal imaging technique is crucial in molecular imaging, depending on the localization and distribution of the imaging target in the pathophysiology of interest (also see next paragraph). Each imaging technology has its advantages and disadvantages. While PET and SPECT have a very high sensitivity for molecular probes marked with radionuclides (nanogram range), the spatial resolution is usually low (PET: 1–2 mm; SPECT: 0.3–1 mm). MRI has a better spatial resolution of 50–250 μm, thereby providing important anatomical information, while molecular probes can be detected in a micro- and milligram range [11].

1.4 Imaging Targets in Vascular Inflammation and Thrombosis

Atherosclerosis is a very complex disease, involving a large number of vascular receptors, cell types, and other processes [1, 5, 8]. Usually, atherosclerosis begins with endothelial activation. Inflammatory endothelial markers such as vascular cellular adhesion molecule (VCAM) and P-selectin can be found, but also platelets adhere at early stages of atherosclerotic plaque formation [2]. This vascular inflammation attracts macrophages, which adhere to the plaque surface and finally migrate into the plaque. In the course of inflammation progression, a lipid core may develop, and proteolysis and apoptosis further promote formation of a lipid-rich necrotic core, neovessels, and formation of a fibrous cap separating this process from the bloodstream. Plaque rupture and exposure of the inflammatory core is a critical event in the pathogenesis of acute vascular syndromes since aggregation of circulating platelets and fibrin can result in immediate vessel closure and therefore acute ischemia in the remote tissue, leading to myocardial infarction in the context of a coronary artery, and stroke when a cerebral vessel such as the carotid artery is occluded. As mentioned above, growth of an atherosclerotic plaque is not a continuous sequence, and also nonobstructive plaques can rupture, particularly where there is an accumulated lipid core and cap thinning associated with local inflammation.

1.5 What to Consider When Performing Imaging Approaches in Vascular Pathologies

Multiple studies have been published over the last years describing molecular imaging approaches in atherosclerosis, involving proof of feasibility or mechanistic studies. Important factors when evaluating such approaches are the following questions: does this study provide a diagnostic value? Does it even allow a prognostic value? Or can it help to guide therapies or evaluate an outcome benefit?

When thinking about imaging studies in atherosclerosis, it is also important to consider the stage at which the imaging is performed, and if the epitopes or processes of interest are exposed superficially, and therefore readily accessible to blood-borne agents (e.g., VCAM or P-selectin), or inside plaque (e.g.,

macrophages, apoptosis). In this context, it is then crucial to choose the sort of imaging technique and the preparation of the contrast agent itself. It is hypothesized that macrophages can phagocytose USPIOs in the bloodstream and enter the plaque carrying the particles and the accumulated USPIO can then provide a signal from "inside" the plaque [12]. By contrast, USPIO would potentially not provide sufficient contrast to image epitopes on the plaque surface in the flowing blood, since the iron load is too low. For this purpose, MPIOs would be more attractive: although they are exposed to high shear stress in the flowing blood, even single particles are detectable by MRI due to the large signal extinction caused [13].

Other thoughts involve the differentiation of noninvasive or invasive imaging. The latter one can be performed with IVUS or optical coherence tomography (OCT), which will be described further below. Finally, the question of whether the imaging is performed on an experimental level in animals or clinically in humans is important. Not all contrast agents described in well-recognized animal imaging studies are necessarily compatible for application in humans due to issues of toxicity or biocompatibility.

1.6 Imaging of Inflammation

In the following section, exemplary studies for imaging of inflammation will be described, using different contrast agent and imaging approaches.

1.6.1 Magnetic Resonance Imaging

As already mentioned above, microparticles of iron oxide (MPIOs) can deliver high payloads of iron toward endothelial epitopes.

In a study published by our group, we performed dual targeting of MPIOs with two different markers of inflammation, imitating leukocyte binding: VCAM and p-selectin [14]. The resulting VCAM/p-selectin-MPIO contrast agent was injected into Apolipoprotein E knockout mice ($ApoE^{-/-}$), which develop atherosclerotic plaques in the ascending aorta and aortic root. Dual-targeted MPIOs, injected intravenously in vivo, bound the aortic root endothelium and were quantifiable by MRI ex vivo. MPIOs were well tolerated in vivo by all mice, with sequestration in the spleen after 24 h. This approach allowed the design of a functional MRI probe for detecting endothelial-specific markers not only in atherosclerosis but in a range of vascular pathologies [15, 16].

Also ultrasmall superparamagnetic iron oxides were used for imaging of endothelial and intraplaque markers of atherosclerosis. In a study by Burtea et al., VCAM-1 and apoptotic cell-targeted peptides were conjugated to USPIO and assessed in $ApoE^{-/-}$ mice by MRI [17]. Plaques enhanced by VCAM-targeted USPIOs contained macrophages concentrated in the cap and a large necrotic core, whereas apoptosis-targeted USPIOs produced a negative enhancement of macrophage-rich plaques inside the plaque.

As discussed above, applications in humans would be desirable, also adding another dimension, such as monitoring of therapeutical effects. One example is the ATHEROMA study, which evaluated the effects of low-dose (10 mg) and high-dose (80 mg) atorvastatin on carotid artery plaque inflammation, as measured by USPIO-enhanced MRI [18]. Twenty patients completed the full 12 weeks of treatment in each group. A significant reduction from baseline in USPIO-defined inflammation was observed in the 80-mg group at both 6 weeks and 12 weeks, whereas there was no visible effect in the low-dose regimen. Interestingly, USPIO were cycled out of the plaque region in between the imaging time points. Unfortunately, to our knowledge, no further clinical studies were performed with this agent, although this molecular imaging strategy could have been a useful biomarker imaging strategy for screening and assessment of therapeutic response to anti-inflammatory interventions in patients with atherosclerotic lesions.

1.6.2 Hybrid Imaging Approaches

Underlying the strategy to combine two different imaging techniques is the idea of getting the best out of each technology. A study by Taqueti et al. investigated the potential of imaging the relationship between markers of inflammatory activation, plaque microvascularization, and vessel wall permeability [19]. Patients with carotid artery plaques were imaged using a multimodality approach combining (1) FDG positron emission tomography (FDG-PET), (2) dynamic contrast-enhanced magnetic resonance imaging (dce-MRI), and (3) histopathology after endarterectomy in 32 subjects with carotid artery stenosis. As a result, plaque regions with active inflammation, as determined by macrophage content and major histocompatibility complex class II expression, showed increased FDG-PET uptake. This correlated with increased microvascularization and permeability, as measured by dce-MRI. Interestingly, the correlation was independent of clinical symptoms and plaque luminal severity, which might therefore be an option for detecting nonobstructive but highly vulnerable/inflamed plaques. Larger studies are desirable to confirm and further strengthen such findings.

Another approach aiming for the detection of ruptured or high-risk coronary atherosclerotic plaques has been described by Joshi et al., combining PET and CT with the radioactive tracers [18]F-sodium fluoride ((18)F-NaF) and [18]F-fluorodeoxyglucose ((18)F-FDG) [20]. Invasive coronary angiography, [18]F-NaF, and [18]F-FDG PET-CT were performed in patients with myocardial infarction and stable angina, and tissue-to-background ratios of culprit and non-culprit coronary plaques of patients with acute myocardial infarction were evaluated. Figure 1.1a shows the PET-CT of a patient with acute ST-segment elevation myocardial infarction with proximal occlusion of the left anterior descending artery on invasive coronary angiography and intense focal 18F-fluoride uptake at the site of the culprit plaque but in remote myocardium. In contrast, the corresponding 18F-fluorodeoxyglucose PET-CT image shows no uptake at the site of the culprit plaque. Another example of a patient with anterior non-ST-segment elevation

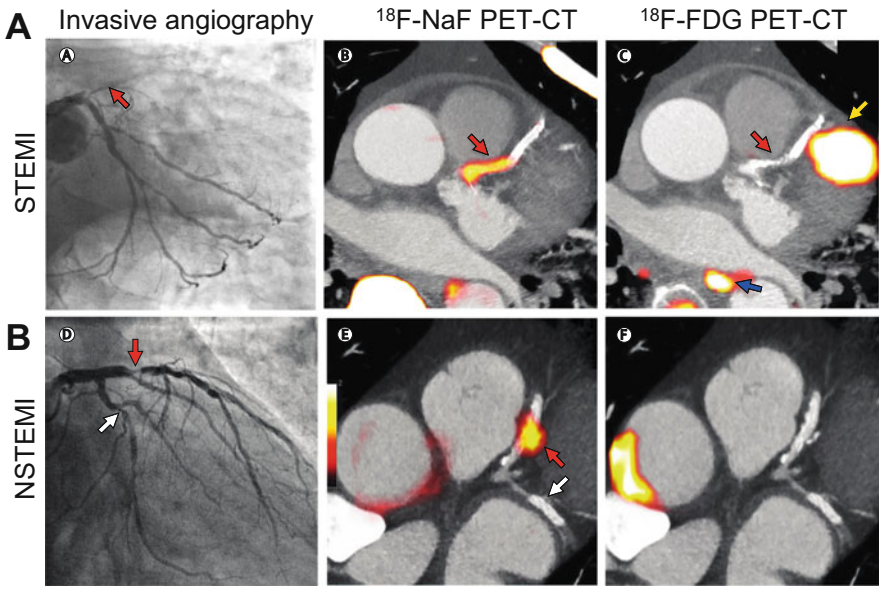

Fig. 1.1 (**a**) PET-CT of a patient with acute ST-segment elevation myocardial infarction with proximal occlusion of the left anterior descending artery on invasive coronary angiography and intense focal 18F-fluoride uptake at the site of the culprit plaque but in remote myocardium. The corresponding 18F-fluorodeoxyglucose PET-CT image shows no uptake at the site of the culprit plaque. (**b**) Example of a patient with anterior non-ST-segment elevation myocardial infarction with a culprit lesion (*red arrow*) and bystander non-culprit lesion demonstrates that only the culprit lesion had increased 18F-NaF uptake on PET-CT; the corresponding 18F-fluorodeoxyglucose PET-CT shows no uptake at either the culprit or the bystander stented lesion

myocardial infarction with a culprit lesion (red arrow) and bystander non-culprit lesion demonstrates that only the culprit lesion had increased 18F-NaF uptake on PET-CT; the corresponding 18F-fluorodeoxyglucose PET-CT shows no uptake at either the culprit or the bystander stented lesion (Fig. 1.1b). In this study, [18]F-NaF PET-CT was the first noninvasive imaging method to identify and localize ruptured and high-risk coronary plaque in a noninvasive way. This is also an interesting and exciting approach of how to combine two imaging modalities and get the best information from each: functional information by PET and anatomical information by CT.

1.7 Imaging of Thrombosis

Thrombosis after plaque rupture involves platelet activation and cross-linking of platelets with fibrin. Both therefore constitute a promising approach for molecular imaging of plaque rupture and atherothrombosis. Platelets are also involved into the inflammatory processes after ischemia caused by reperfusion.