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Invited critical review

Laboratory medicine for molecular imaging of atherosclerosis

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ABSTRACT

Atherosclerotic plaques are the main cause of life threatening clinical endpoints like myocardial infarction and stroke. To prevent these endpoints, the improved early diagnosis and treatment of vulnerable atherosclerotic vascular lesions are essential. Although originally applied for anticancer treatment, recent advances have also showed the considerable potential of nanotechnology for atherosclerosis. Otherwise, one domain of laboratory medicine is the investigation of new biomarkers. Recent research activities have identified the usability of biomarker-targeted nanoparticles for molecular imaging and pharmacologic modification of vulnerable atherosclerotic lesions leading to myocardial infarction or stroke. These investigations have established a new research interface between laboratory medicine, nanotechnology, cardiology/neurology, and radiology.

In this review, we discuss inflammatory pathophysiologic mechanisms and biomarkers associated with a vulnerable atherosclerotic plaque phenotype. Further, we will emphasize cardiovascular relevant functionalized nanoparticle biomarker constructs which were developed within the cooperation interface between Laboratory Medicine (anti-inflammatory biomarkers), Nano-Medicine (nanoparticle development), and Radiology (molecular imaging).

Contents

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1. Introduction

Despite diagnostic and therapeutic advances, cardiovascular endpoints are still leading causes of mortality worldwide. This is primarily due to the increasing prevalence of atherosclerosis (AS), frequently associated with the metabolic syndrome (MetS), usually caused by a sedentary, obesogenic lifestyle. Atherosclerosis is a sub-acute inflammation around lipid deposits in the vascular wall, characterized by the infiltration of macrophages and T cells interacting with one another and with the arterial wall cells [1]. The adaptive and innate immune systems are involved in the generation of vulnerability within the plaque scenario [2–6]. Currently, AS tends to be diagnosed too late at the advanced stages of the disease, either by directly measuring the degree of stenosis or by evaluating the effect of arterial stenosis on organ perfusion [7].

Recent advances in imaging techniques have provided new options for improved visualization and monitoring of AS-lesions' dynamics of progression or regression [8]. Nevertheless, a reliable, cost-effective, non-invasive technique to detect different stages of AS for the applicable, clinical characterization of AS-plaques has yet to be developed [9].

Nanomedicine applies nanotechnology for the diagnosis, therapy, and monitoring of diseases. Medical nanoparticles (NP) are typically between 1 and 300 nm in size, and resemble in scale macromolecules like proteins and DNA. Nanoparticles can consist of organic material (e.g. spherical phospholipid bilayer self-assemblies — so called liposomes), polymeric, inorganic or metallic materials (e.g. iron oxide, gold) or combinations thereof [10]. Usually, NP have a high surface-to-volume ratio, which is well suited for coating the surface with a variety of molecules, e.g. for specific targeting of pathologic key processes [11]. The investigation of new biomarkers for diagnosis, monitoring, and control of cardiovascular disease (CVD) is an exciting challenge for laboratory medicine. These biomarkers can be potentially useful molecules for coating NP to generate a selective targeting capacity for pathologic processes. Herein, we discuss these aspects in the context of improved diagnosis and treatment of the two most important clinical endpoints of AS—myocardial infarction and stroke.

1.1. Historical background of nanomedical applications

Liposomal formulations of chemotherapeutics (e.g. doxil) belong to the first clinically approved drugs based on NP. Subsequently, other nanotherapeutics have been developed, like liposomal amphotericin B for fungal infections, liposomal daunorubicin and albumin-bound NP delivering paclitaxel to treat breast cancer. The encapsulation of these cytostatic compounds in NP results in improved pharmacokinetics compared to the free drug and decreases both the drug's clearance from the blood stream as well as cardiac and liver toxicity. These effects may also be useful for a more effective treatment of atherosclerotic lesions. Apart from therapeutic application, NP have been labeled or packed with the following small molecules, chelated ions, metals or nanocrystals for visualisation by diagnostic imaging:

(1) gadolinium (Gd^{3+}) chelates or iron oxide for magnetic resonance imaging (MRI) [12,13]
(2) electron dense elements (gold or bismuth) for X-ray and computed tomography [14]
(3) radiolabels (^{18}F,^{64}Cu, ^{89}Zr) for positron emission tomography (PET) [15–17]
(4) ^{111}In for single-photon emission computed tomography (SPECT) imaging [18], and
(5) fluorophores or quantum dots for optical imaging [19].

To summarize, although primarily applied in cancer, nanomedicine is now likely to play a substantial role in cardiovascular research, particularly for improved diagnosis and therapy of AS lesions underlying myocardial infarction and stroke. The biomarker research of laboratory medicine may substantially contribute to this important development.

2. Atherosclerosis, inflammation and vulnerability

Atherosclerosis is the major cause of morbidity and mortality in CVD, and represents a substantial economic burden [20]. Atherosclerotic plaques from coronary arteries cause fatal clinical endpoints after myocardial infarction, and those from carotid arteries are responsible for ischaemic stroke [21]. Chronic systemic immune-mediated inflammation is a key contributor to the pathologic process. The involvement of the toll-like receptors, TLR2 [22], TLR4 [23] and TLR7 [6] underlines the role of the innate immune response in AS. Further, endogenous danger-associated molecular patterns (DAMPs) activate the innate immune response. The DAMP proteins S100A8 and S100A9 from the S100 calgranulin family are of interest in this context. Both form a heterodimer (MRP8/14 or calprotectin), and are constitutively expressed in myeloid cells. Usually increased by traditional cardiovascular risk factors like smoking, obesity, hyperglycemia, and dyslipidemia, S100A8/A9 is an endogenous ligand of the toll-like receptor 4 (TLR4) and the receptor for advanced glycation end products (RAGE). It has been shown to correlate in humans with the extent of coronary and carotid atherosclerosis, and most importantly with a vulnerable plaque phenotype [23]. Thus, S100A8/A9 may become an interesting laboratory biomarker for the development of vulnerability in AS.

2.1. Early stages of AS lesions

The damage of endothelial cells is an important first step in AS. It leads to an increased penetration of lipoproteins into deeper vascular wall layers, induces the expression of chemotactic chemokines/cytokines and adhesion molecules, and results in enhanced recruitment and accumulation of monocytes within the vascular wall [24]. The monocytes transform into macrophages and foam cells by ingestion of apolipoprotein B containing low-density lipoprotein (LDL). Effector cells of the adaptive immune response (preferentially Th1/Th17 lymphocytes) and lipoproteins accumulate in the subendothelial space [3]. Either this sequential process is self-limited by the resolution of inflammation, or it progresses to a complex scenario characterized by ingression of aggressive macrophage subtypes with foam cell development [25,26], cell apoptosis, tissue necrosis around the lipid core, bleeding, activation of metalloproteinases and neovascularization. This is accompanied by a sustained activation of the adaptive immune system (i.e. increased $CD4^{+}$Th1, reduced T-regulatory cells), less interleukin-10 production [26], and increased infiltration of mature dendritic cells [27] over a period of years or even decades [28,29].

2.2. Advanced stages of AS lesions

Advanced AS lesions usually contain an extended area of lipids and necrotic cells. In normal vessels the intima is supplied with nutrients by diffusion from the lumen, distal regions from the vasa vasorum. With progression of the AS lesion, the intima thickens and local hypoxia arises. Within this scenario, neovascularization acts as a compensatory mechanism. Nevertheless, increased micro vessel density has been suggested as being critically involved in the occurrence of clinical endpoints in the context of intraplaque mast cell accumulation [30]. Thus, intensity of lipid deposition, neovascularization, increased density of mast cell chymase and tryptase, increased macrophage content, and decreased collagen deposition are important destabilizing factors [6,31]. Plaque neovascularization is strongly stimulated by inflammation. These vessels are fragile and lack mural cells, and they own ineffective endothelial cell junctions [32], which contributes to destabilization. Further, AS plaque calcification is an important issue for vulnerability. Nevertheless, its true impact remains controversial [33,34]—even a stabilizing role has been considered [34]. Albeit coronary artery calcium (CAC) score values have turned out useful to define the generic risk of acute coronary events in a population, the vulnerable plaque that needs to be treated to prevent an acute event cannot be identified by CAC scores [34].