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Review

Nanomedicine for the molecular diagnosis of cardiovascular pathologies

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ABSTRACT

Predicting acute clinical events caused by atherosclerotic plaque rupture remains a clinical challenge. Anatomic mapping of the vascular tree provided by standard imaging technologies is not always sufficient for a robust diagnosis. Yet biological mechanisms leading to unstable plaques have been identified and corresponding biomarkers have been described. Nanosystems charged with contrast agents and targeted towards these specific biomarkers have been developed for several types of imaging modalities. The first systems that have reached the clinic are ultrasmall superparamagnetic iron oxides for Magnetic Resonance Imaging. Their potential relies on their passive accumulation by predominant physiological mechanisms in rupture-prone plaques. Active targeting strategies are under development to improve their specificity and set up other types of nanoplatforms. Preclinical results show a huge potential of nanomedicine for cardiovascular diagnosis, as long as the safety of these nanosystems in the body is studied in depth.

1. Introduction

Atherosclerosis is a slowly progressing pathophysiological process characterized by the formation of lipid-rich plaques in the intima of medium and large arteries. It is a major burden in Western societies principally induced by sedentism and rich fat diets [1]. Genetic factors play also an important role in the evolution of this disease [2]. The development of atherosclerosis itself is generally not fatal. However, atherosclerotic lesions became dangerous when they develop a thin fibrous cap and a large necrotic core [3]. In these conditions, plaques may break exposing thrombogenic substances to the circulation driving the formation of an intraluminal thrombus. Complications induced by thrombosis, such as ischemic stroke and myocardial infarction (also referenced as acute clinical events (ACE)), are and will remain the leading cause of death in the world with more than 17 million of deaths per year [4].

Identifying people at risk to develop rupture-prone atherosclerotic plaques and predicting the rupture are therefore essential for a better care. Clinical methods performed to assess plaque evolution, such as Magnetic Resonance Angiography (MRA) and Angiography performed with Computed Tomography (CT) imaging, provide morphological information about the intraluminal stenosis generated by plaque growing. However, it has been observed that an advanced degree of stenosis is not necessarily characterizing unstable plaques.

Progresses have been made in biological understanding of phenomena leading to plaque rupture. Molecular markers more likely to be expressed in high-risk plaques have been identified, as well as some of their specific ligands. Targeting and imaging these biomarkers with contrast agents would enable to better identify plaque components and to provide information about the activation state conducive to plaque rupture.

Technological improvements in the field of medical imaging provide equipment with better resolution and sensitivity. Molecular imaging is now conceivable, as far as the specificity of the targeting method and the sensitivity of the imaging modality ensure injection of safe doses of contrast agents. To that end, nanoplatforms combining existing contrast agents with targeting units have been developed. They show exciting properties related

Abbreviations: ACE, acute clinical event; MRA, magnetic resonance angiography; CT, computed tomography; AAA, Abdominal Aortic Aneurysm; MRI, Magnetic Resonance Imaging; ox-LDL, oxidized Low Density Lipoproteins; SMC, smooth muscle cell; ApoE−/− mice, Apolipoprotein E Knockout mice; HDL, High Density Lipoprotein; PLGA, Poly(lactic-co-glycolic acid); PVA, polyvinyl alcohol; PEG, polyethylene glycol; MP, microparticle; MI/R, myocardial ischemia/reperfusion

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to their nanometric scale and to their potential for functionalization. Furthermore, they have great surface properties and can be tuned to interact preferentially with specific cells and plaque components.

This review highlights the relevance of nanomedicine for a personalized and robust diagnosis of atherosclerosis as well as for other types of lesions, such as Abdominal Aortic Aneurysms (AAA) and transient ischemic events. It describes the nanosystems in preclinical development and in clinical use for Magnetic Resonance Imaging (MRI), Nuclear Imaging and X-ray Computed Tomography (CT).

2. Molecular targets of cardiovascular pathologies

Among the different molecular and cellular targets identified in cardiovascular pathologies, some have been used for molecular imaging and are represented in Fig. 1.

Biological mechanisms of plaque evolution to rupture have been largely described [5]. Cholesterol charged Low-Density Lipoproteins (LDL) enter the intima where they become oxidized (ox-LDL). Activated endothelial cells express specific markers such as P- and E-selectins, VCAM-1 and ICAM-1 that allow for recognition and infiltration of monocytes. Monocytes differentiate into macrophages and accumulate in the plaque. Smooth muscle cells (SMCs) migrate into the intima and become activated, losing their stretching phenotype. Macrophage and SMCs become charged in ox-LDL generating foam cells. In active plaques, a fibrous cap forms made of SMCs and collagen fibers. Remodeling happens affecting the size of the intima outwards the vessel wall, which cannot be assessed by conventional imaging techniques. Apoptotic events and necrotic core appear in a hypoxic environment inducing micro-vessels development (angiogenesis) [6].

The mechanisms of plaque development being understood, different molecular markers have been identified, among which: endothelial targets (VCAM-1, ICAM-1, E- and P- selectins) [7], macrophage targets (scavenger receptor class A (SR-A) and integrin MAC-1), fibrous cap and extracellular matrix (collagen types I, III, IV), apoptosis (expression of phosphatidylserine) and angiogenesis [8]. For preclinical studies, animal models have been developed to mimic the atherosclerotic lesions, such as Apolipoprotein E deficient mice (ApoE−/− mice) [9]. This model is the most common one but it shows some limitations. Although plaques become highly activated, their rupture is not observed.

E- and P- selectins expressed by activated endothelial cells are also relevant as molecular targets for the diagnosis of AAA and transient ischemic events. These pathologies would also benefit from molecular imaging. In clinic, the size of an aneurysm is the only criterion to predict its evolution and to assess risk for rupture. However, its expansion could be better predicted with complementary information about its activation state [10]. In addition to endothelial activation, the presence of a forming thrombus is especially worth detecting. Circulating platelets become activated and accumulate onto the affected endothelium. A fibrin mesh forms and platelets and red blood cells accumulate at site of injury. Detection can be achieved by targeting fibrin, activated factor XIII and P-selectin expressed by activated platelets. In case of transient ischemic event, physicians lack tools to precisely evaluate the extent of the damage. Yet the endothelium remains highly activated for a few hours after the event [11]. This phenomenon is referred to as ischemic memory.

A promising strategy to achieve the detection of the described biomarkers is the design of nanoplaftorms through their high potential for functionalization and their compatibility with existing imaging technologies.

3. Nanoplaftorms for molecular imaging of cardiovascular pathologies

Three main types of platforms have been developed for molecular imaging of cardiovascular pathologies: lipid-based, polymer-based and inorganic nanoparticles.

Lipid-based nanoparticles are composed of a phospholipid assembly. Phospholipids are amphiphilic components. Under aqueous conditions, they organize into structures, such as micelles, liposomes and micro-emulsions. Micelles are made of a monolayer of lipids surrounding a hydrophobic core. They are generally smaller than 50 nm. Liposomes are made of a phospholipid bilayer enclosing a hydrophilic core. Their size may reach several hundreds of nm. Micro-emulsions consist in micelles containing hydrophobic nanodroplets and are usually of a few hundreds of nm. Natural High-Density Lipoproteins (HDL) or HDL-like synthetic particles are also of great interest as they are naturally entering LDL-rich plaques and show the advantages of being endogenous and entirely biodegradable [12]. They are micelles made of extracted or synthesized lipoproteins, mainly apolipoproteins apo A-I and apo A-II. Their size stands around 5–17 nm. According to their biocompatibility and their versatility, lipid-based nanosystems have led to numerous formulations.

Synthetic and natural polymers have also been studied for the design of nanostructures more resistant to mechanical constraints than lipid-based systems. Different techniques, such as nanoprecipitation [13] and emulsion polymerization [14], enable to formulate polymer nanoparticles. According to the polymer nature and to the synthesis parameters, polymer platforms can be tuned in terms of size, porosity and hydrophobicity. Poly(lactic-co-glycolic acid) (PLGA), FDA-approved polymer, and Polyvinyl alcohol (PVA) are biodegradable polymers widely studied for nanomedical research [15,16]. Polysaccharides show also promising properties for cardiovascular applications. Among them, chitosan and fucoidan present affinity for fibrin and P-selectin respectively [17].

Inorganic nanoparticles are composed of an inorganic core with imaging properties, generally coated with a polymer shell. In particular, magnetic nanoparticles have been developed for MRI and metal nanoparticles for CT. The polymer coating improves colloidal stability and particle biocompatibility. Alternatively, other coatings include small molecules, as bisphosphonates, and phospholipids. For these three types of platforms, Poly(ethyleneglycol) (PEG), a hydrophilic biocompatible and biodegradable polymer, has been widely used as a coating in order to prevent nanoparticles aggregation and improve their pharmacokinetic properties [18].

4. Magnetic Resonance Imaging

4.1. Imaging modality and corresponding contrast agents

Magnetic Resonance Imaging (MRI) provides images with a very good resolution (from 25 μm to 1 mm) and a precise localization in the body while ensuring no exposure to ionizing radiations. MRI relies on the relaxation of hydrogen nuclei present in abundance in tissues. Under a strong and uniform magnetic field, typically from 1.5 to 3 T, the intrinsic magnetic moments of hydrogen nuclei of scanned tissues align. Resonance of hydrogen nuclei is then obtained via the application of an oscillating magnetic field. Each time the oscillating field is switched off, magnetic moments return to their equilibrium position at a given rate called relaxation. Two different relaxation times characterize a nucleus: its longitudinal relaxation time (T1) and its transversal one (T2 or T2*). Recording these relaxation times give information about