Nanoparticle-Mediated Drug Delivery System for Cardiovascular Disease

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Summary

Administration of drugs and other therapeutic agents has been the central strategy of contemporary medicine for cardiovascular disease. The use of a drug delivery system (DDS) is always demanded to enhance the efficacy and safety of therapeutic agents, and improve the signal-to-noise ratio of imaging agents. Nano-scale materials modify in vivo drug kinetics, depending on (patho)physiological mechanisms such as vascular permeability and incorporation by the mononuclear phagocyte system, which constitute ‘passive-targeting’ properties of nano-DDS. By contrast, an ‘active-targeting’ strategy employs a specific targeting structure on nano-DDS, which binds to the target molecule that is specific for a certain disease process, such as tumor specific antigens and the induction of adhesion molecules. In this review, we summarize recent studies that applied nano-DDS for the diagnosis and treatment of cardiovascular disease, especially focusing on atherosclerosis and myocardial ischemia-reperfusion (IR) injury. Pathophysiological changes in atherosclerosis and myocardial IR injury are successfully targeted by nano-DDS and preclinical studies in animals showed positive effects of nano-DDS enhancing efficacy and reducing adverse effects. The development of nano-DDS in clinical medicine is keenly being awaited. (Int Heart J 2014; 55: 281-286)

Key words: Nanotechnology, Atherosclerosis, Myocardial infarction, Ischemia reperfusion, Inflammation

Nanoparticle-Mediated Drug Delivery Systems

Administration of drugs and other therapeutic agents has been the central strategy of contemporary medicine, based on the concept that a certain disease is caused by a formation of abnormal or diseased cells within healthy organs and the body. In order for drugs to affect disregulated organs and cells, drugs need to overcome physiological barriers, namely circulation to organs, and tissue to cells, and reach target molecules within the cells. On the other hand, all drugs possess potential toxicity that may limit their safe dose and thereby therapeutic efficacy. Targeting drugs to diseased organs/cells may reduce the potential risks of adverse effects; therefore, the use of a drug delivery system (DDS) is always needed to enhance the efficacy and safety of therapeutic agents, and overcome any drawbacks of the agents, such as toxicity, low water solubility, poor bioavailability, and low organ specificity. Moreover, targeting delivery is a desirable property for diagnostic purposes as it improves the signal-to-noise ratio and optimizes sensitivity and specificity.

Recent application of nanotechnology to medicine has developed nanoparticle-mediated DDS (nano-DDS), which modifies the in vivo kinetics of therapeutic and diagnostic agents. One of the most important motives for nano-DDS is drug targeting, which may utilize physiology and pathophysiological properties unique to certain disease processes. Nano-DDS can be composed of a variety of materials and structures, including lipids to form micelles or liposomes, polymers, dendrimers, carbon nanotubes, and metallic nanoparticles such as crystalline iron oxide and gold nanoparticles (Figure 1). Here we describe selected examples of nano-scale materials tested as nano-DDS. Micelles are formed from lipids and other amphiphilic artificial molecules such as polymers. Micelles self-assemble in aqueous solution and may incorporate hydrophobic therapeutic agents to overcome solubility problems. The size (usually 10-100 nm in diameter) and the enclosed space are more confined to those of liposomes (Figure 1A). Liposomes mainly consist of phospholipids that form bi-layers with an aqueous phase inside, and are heterogeneous in size, often ranging from a few hundreds to thousands of nanometers in diameter. Liposomes are the most extensively tested nano-DDS in basic and clinical medicine with United States Food and Drug Administration (FDA) approval. Chemicals, nucleotides, and also crystalline metals are incorporated in liposomes (Figure 1B). Currently, two polymers, polylactide (PLA) and poly(lactide-co-glycolide) (PLGA), are used for the synthesis of FDA-approved polymeric biodegradable nano-DDS. PLGA polymers may incorporate hydrophilic and hydrophobic therapeutic agents including chemicals and nucleotides by emulsion solvent diffusion methods, and are being tested for intractable diseases including cardiovascular disease (Figure 1C). Dendrimers are highly branched macromole-
Intravital kinetics of nano-DDS may be diverse; their behavior within the biological environment is affected not only by size, but also by their chemical makeup and morphology. However, the most important determinant of the physiological behaviors of nano-DDS is the size, as discussed below.

Physiological Behavior of Nano-DDS

Nano-sized materials (10–300 nm in diameter) tend to remain in circulation avoiding renal excretion, which is a primary feature of nano-DDS. While circulating in the blood stream, nano-DDS extravasates from the vasculature with enhanced permeability, such as angiogenic vessels in tumors, and vessels in organs after ischemia-reperfusion, which is an important mechanism that affects tissue distribution of nano-DDS.\(^\text{2,3,24-26}\)

The neovasculature in tumors lacks functional lymphatic vessels as well as enhanced vascular permeability, causing an accumulation of nano-DDS in the tumor microenvironment.\(^\text{31}\) This phenomenon is referred to as enhanced permeability and retention (EPR) effects of nano-DDS.\(^\text{27}\)

Recognition and incorporation by the mononuclear phagocytic system (MPS, also called the reticuloendothelial system), namely neutrophils, monocytes, and macrophages in the blood, liver, spleen, and lymph nodes is also a common physiological behavior for nano-DDS, which may affect the blood circulating time and tissue/cell distribution.\(^\text{22-24}\) One of the first clinically approved nano-scale DDS therapies was a liposomal formulation of doxorubicin, a cytotoxic drug used for cancer chemotherapy. During its development, encapsulation of doxorubicin in liposomes prolonged its blood half-life compared to the free drug, but this was found to be unsatisfactory because of entrapment by MPS.\(^\text{32}\)

The addition of polyethylene glycol (PEG) to the surface of nano-DDS was shown to reduce the recognition of MPS, and in the case of doxorubicin liposomes, the addition of PEG reduced the clearance from the bloodstream, as well as cardiotoxicity, which is a major adverse effect.\(^\text{33}\) On the other hand, incorporation into nano-DDS itself is one of the mechanisms of drug delivery, especially when treating an inflammatory disease, such as atherosclerosis. Several studies employ this mechanism for the imaging and treatment of atherosclerosis, target-ing inflammatory monocytes/macrophages in atherosclerosis.\(^\text{31}\)

The above-mentioned mechanisms underpin a ‘passive-targeting’ of nano-DDS on diseased organs or MPS. By contrast, an ‘active-targeting’ strategy employs a specific targeting structure on nano-DDS, which binds to the target molecule that is specific for a certain disease process. One good example is tumor-specific expression of folate receptor, which is target-ed by addition of folate to the surface of nano-DDS.\(^\text{33,34}\) For the treatment of cardiovascular disease, vascular endothelial cells can be targeted by an antibody for platelet endothelial cell adhesion molecule (PECAM-1). Liposomes loaded with a superoxide dismutase mimetic were conjugated with PECAM-1 antibody, which increased the delivery of liposomes to the pulmonary vasculature, and successfully enhanced the anti-inflammatory effects against endotoxin-induced acute lung injury.\(^\text{35}\) Vascular cell adhesion molecule-1 (VCAM-1) is another molecule for targeting vascular endothelial cells.\(^\text{36}\) There are still numerous opportunities for an ‘active-targeting’ strategy to find specific target molecules for a certain disease process, the effectiveness of which may be investigated in future studies.

Nano-DDS for Atherosclerosis

Atherosclerosis is one of the oldest diseases and was even present in ancient times, and atherosclerotic CVD, such as acute myocardial infarction (MI) and stroke, are still major causes of death and disability worldwide. Atherogenesis begins as an endothelial dysfunction; when subjected to oxidative, hemodynamic, or biochemical stimuli (from smoking, hypertension, or dyslipidemia) and inflammatory factors, endothelial cells change their permeability and the expression of adhesion molecules to promote the recruitment of circulating monocytes and cholesterol-containing LDL particles. Inflammation and biochemical modifications ensue, causing endothelial and smooth muscle cells to proliferate, produce extracellular matrix molecules, and form a fibrous cap over the developing atheromatous plaque. Narrowing of the arteries by atheromatous plaque limits blood flow causing ischemic vascular diseases such as angina pectoris, whereas the rupture of a fibrous cap causes abrupt cessation of blood flow via thrombosis, resulting in end organ damage such as AMI. A series of pathological analyses in patients with sudden coronary deaths showed that ruptured coronary lesions typically have large necrotic cores and a disrupted fibrous cap infiltrated by macrophages with an expression of matrix metalloproteinases (MMP), suggesting that inflammatory macrophages contribute to the destabilization and rupture of atherosclerotic plaques, resulting in a thrombotic occlusion of the coronary artery and AMI. Among the molecular and cellular mechanisms of long-term atherogenesis leading to plaque destabilization, (1) enhanced vascular permeability, (2) expression of adhesion molecules in endothelial cells, (3) accumulation of inflammatory monocytes (Ly6Chigh CCR2+ in mice, CD14+CD16− in humans) in macrophages, and (4) expression of proteases that facilitates plaque destabilization are potential mechanisms for drug delivery, and thereby targets for imaging and therapeutic intervention of atherosclerotic cardiovascular disease including coronary artery disease.

Computed tomography (CT) is a modality which has been extensively used for the imaging of coronary arteries in clinical practice. Recent advances in multi-detector CT that can simultaneously acquire a volume of images have enabled us to acquire a complete coronary angiogram in less than a minute. Iodine-containing contrast agents are used to image coronary arterial lumens, identifying the narrowing of coronary lumens by atherosclerotic lesions. Recent clinical studies also describe ‘unstable plaque’ by coronary CT, as defined by a luminal narrowing that is associated with expansive or positive vessel remodeling (PR), and low-attenuation plaques (LAP), which suggests a high risk for plaque rupture and acute coronary syndrome. MRI produces tomographic images with high soft-tissue contrast and spatial resolution, and is another important imaging modality for cardiovascular disease. SPIO are strong nanoscale contrast enhancers for MRI, and because of their remarkable biocompatible and biodegradable properties, several SPIO particles with diverse sizes, coatings, and targeting abilities have been applied for imaging of the inflammatory process of atherosclerosis and myocardial infarction. Macrophages internalize SPIO, altering the local magnetic field and thus producing the T2 shortening effect as visualized by signal reduction. Monocrystalline iron oxide nanoparticles (MION)-47 have an approximate 5-nm diameter core of SPIO coated with an approximate 10-nm-thick dextran layer and have a long blood half-life, which facilitates their accumulation in macrophages of atherosclerotic plaques. Incorporation of MION-47 in macrophages in atherosclerotic lesions was confirmed histologically in a rabbit model. Increased Fe content causes loss in T2 signals, which enables negative imaging of macrophage-rich lesions. Targeting VCAM-1 was also tested for the imaging of atherosclerotic lesions in a mouse model, and showed enhanced delivery of SPIO by VCAM-1 targeting.

Our group has tested polymeric PLGA nanoparticles as a nano-DDS for the treatment of atherosclerotic plaque destabilization. PLGA is a biodegradable material and is approved by the FDA and the European Medicine Agency for various DDS in human clinical use. In atherosclerotic ApoE-deficient mice fed a high fat diet and infused with angiotensin II, neutrophils and monocytes in the peripheral blood and the aorta incorporated FTTC-loaded PLGA nanoparticles (FTTC-NP) 2 hours after injection, determined by flow cytometric (FCM) analysis (Figure 2A). FTTC-NP accumulated in atherosclerotic lesions in the aortic arch (Figure 2B), and fluorescence microscopy analysis revealed that FTTC signals are observed mainly in the macrophages of atherosclerotic plaques, which is partially blocked by depletion of monocytes by clodronate, suggesting that PLGA nanoparticles are delivered to atherosclerotic lesions partly through monocyte/macrophage phagocytosis, and directly via enhanced permeability in the lesions. Weekly intravenous treatment with PLGA nanoparticles containing the HMG-CoA reductase inhibitor pitavastatin reduced circulating inflammatory Ly6Chigh monocytes, macrophage infiltration to the atherosclerotic lesions in the aortic root, and plaque destabilization in the brachiocephalic arteries (Figure 2C, D). Another group tested liposome-dependent delivery of siRNA against chemokine receptor CCR2, and showed successful delivery to spleen, bone marrow, and liver, and the inhibition of monocyte/macrophage recruitment to the aorta and atherosclerotic lesions.

The above-mentioned studies consistently showed successful delivery of nano-DDSs including crystalline metal, polymers, and liposomes to monocytes/macrophages, mainly through physiological entrapment by MPS, and these nano-DDSs can be applied to both the imaging and treatment of atherosclerosis.

Nano-DDS for Acute Myocardial Infarction and Ischemia-Reperfusion Injury

Acute MI is a major cause of death and heart failure worldwide. In patients with ST-segment elevation acute MI (STEMI), early reperfusion therapy is a standard strategy to limit MI size; however, recent cohort studies suggest that the
Figure 2. Nano-DDS for atherosclerosis. A: Flow cytometry of circulating leukocytes 2 hours after intravenous injection of PLGA nanoparticles encapsulated with FITC (FITC-NP). The histograms demonstrate FITC uptake by monocytes in the blood and the aorta. B: Fluorescent and light micrographs of isolated aortic arch 24 hours after intravenous injection of FITC-NP or saline. C, D: Photomicrographs of atherosclerotic plaques in aortic root (C) and brachiocephalic arteries (D) stained with EVG or Mac3. Arrows indicate disrupted/buried fibrous caps.

Figure 3. Nano-DDS for myocardial IR injury. A: Fluorescence reflectance imaging of the sections of heart in sham operated mouse or myocardial IR mouse. Accumulation of indocyanine green-loaded nanoparticle was noted in an IR heart after intravenous treatment at the time of reperfusion. B: Flow cytometric analysis in the leukocytes of an IR heart showed the incorporation of FITC-loaded nanoparticles was noted in macrophages and neutrophils.

Incorporation by circulating monocytes and other MPS is an established phenomenon after myocardial injury, and several animal studies have suggested a role of inflammation as a therapeutic target in IR injury. However, several clinical trials on pharmacological cardioprotection for myocardial IR injury have failed to demonstrate a positive impact on clinical outcome in STEMI patients. One possible explanation for the failure of current clinical trials is an insufficient drug delivery during a limited interventional time window, while administered at the time of reperfusion. Therefore, from a clinical perspective, it is feasible to apply an effective DDS that facilitates delivery to the sites of IR injury during reperfusion, a clinically feasible time point.

Nano-DDS may accumulate in injured tissues, including IR myocardium, where vascular permeability is enhanced. Incorporation by circulating monocytes and other MPS is another mechanism targeting inflammation after myocardial injury. Thus, nano-DDS may be feasible for myocardial IR injury targeting ischemic myocardium and inflammatory monocytes. Takahama, et al have tested PEGylated liposome-dependent delivery of adenosine during myocardial reperfusion, and found that liposomes attained a higher adenosine concentration in the ischemic myocardium and showed superior cardioprotection and less systemic hypotensive effect compared with free adenosine in a rat model. Leuschner, et al have tested liposome-dependent delivery of siRNA against CCR2, and showed successful delivery to spleen, bone marrow, and liver, and the inhibition of monocyte/macrophage recruitment to the heart after IR. Treatment with siRNA-CCR2 successfully reduced MI size.

We have examined the efficacy of PLGA nanoparticles as a DDS for myocardial IR injury. In a mouse model of myocardial 30-minute ischemia-reperfusion, PLGA containing indocyanine green-loaded PLGA nanoparticles was traced with fluorescence reflectance imaging. PLGA nanoparticles were found exclusively in the ischemic myocardium (Figure 3A). Flow cytometric analysis showed the incorporation of FITC-
Cardiovascular diseases, namely atherosclerosis and myocardial IR injury. Enhance therapeutic efficacy through targeting specific mechanisms of myocardial IR injury.

1. Nano-DDS for cardiovascular disease

Figure 4. Perspective of nano-DDS-mediated treatment for acute coronary syndrome. Nano-DDS-mediated treatment for cardiovascular disease comprises 1) timely intravenous injection of nano-DDS, 2) nano-DDS-dependent drug delivery to circulating monocytes, atherosclerotic arterial wall and macrophages, and ischemic myocardium, and 3) therapeutic goals including atherosclerotic plaque stabilization, reduced myocardial infarct size, and improvement of patient prognosis.

Loaded PLGA nanoparticles in macrophages and neutrophils in the IR heart (Figure 3B). Nano-DDS-dependent delivery to inflammatory cells and ischemic myocardium is a desirable property for the treatment of myocardial IR injury. PLGA nanoparticles and other nano-DDS including liposomes will be tested in clinical trials in the future to test whether they will enhance therapeutic efficacy through targeting specific mechanisms of myocardial IR injury.

Summary and Clinical Perspective

In this review, we summarized the properties of selected nano-DDSs and their preclinical studies in animal models of cardiovascular diseases, namely atherosclerosis and myocardial infarction. Current applications of nano-DDS for cardiovascular disease utilize mainly 2 major mechanisms of drug delivery, enhanced vascular permeability and incorporation by MPS, which underpins enhanced drug delivery for circulating monocytes, atherosclerotic arterial wall and macrophages, and ischemic myocardium. These properties of nano-DDS may be applicable for the treatment of acute coronary syndrome (coronary plaque destabilization and acute myocardial infarction) (Figure 4).

Although preclinical studies have reported nano-DDS have therapeutic effects on atherosclerotic plaque stabilization and myocardial IR injury, there is a wide variety of opportunities to combine nano-DDSs and various therapeutic agents, including chemicals, nucleotides, peptides, and others, which may expand the potential of current pharmacotherapy for several cardiovascular diseases. Possible application of nano-DDS in other cardiovascular diseases may include pulmonary hypertension, vein graft disease, and therapeutic neovascularization for clinical limb ischemia and coronary stents. We have started a phase IIa investigator-initiated clinical trial in Kyushu University Hospital to test the efficacy of PLGA nanoparticle-mediated delivery of pitavastatin in patients with critical limb ischemia (UMIN000008011). Future clinical trials conducted in the next decade may prove the safety and efficacy of nano-DDS for cardiovascular diseases.

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