REVIEW

NANOPARTICLES IN DELIVERY OF CARDIOVASCULAR DRUGS

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ABSTRACT

Everything in nature is built upward from the atomic level to define limits and structures to everything. Nanomedicines marked the field of medicine from nanobiotechnology, biological micro-electromechanical systems, microfluidics, biosensors, drug delivery, microarrays to tissue microengineering. Since then nanoparticles has overcome many challenges from blood brain barrier to targeting tumors. Where solid biodegradable nanoparticles were a step up liposome, targeting nanoparticles opened a whole new field for drug delivery. In this article, we attempt to discuss how the pioneered technique is serving in the drug delivery to cardiovascular system and how with the manipulation of their properties, nanoparticles can be made to fulfill desired function. Also how nanocarriers are improving molecular imaging to help improve diagnosis and treatment of cardiovascular disease is focused in this article.

Keywords: Nanotechnology, nanoparticles, drug delivery, cardiovascular drugs and molecular imaging.

INTRODUCTION

A highly developed brain supporting system that isolates and protects the brain from the intrusion of unwanted substances which circulate in the blood stream, also limits the delivery of therapeutic agents into the brain is what known as Blood Brian Barrier (BBB). Many of the drugs which act on the central nervous system (CNS) either cannot cross the BBB in sufficient quantity, degrade soon after they reach the brain or are pumped out of the brain by Pglycoprotein (P-gp) (Chen Y *et al.,* 2004). Scores of ways have been designed to overcome BBB, including invasive technique, neurosurgery-based infusion or implants to deliver drug behind the BBB, physical or chemical disruption of the BBB via osmotic shift, biochemical and immunological changes (Pardridge, 2001) and non-invasive approaches (based on the bioavailability or plasma area under the concentration curve (AUC) of the drug) (Chen Y *et al.,* 2004). What improved drug delivery to the CNS to an enormous extent was the introduction of liposomes as drug carrier (Gregoriadis, 1973, 1976).

Liposomes are a composition of amphiphilic phospholipids and cholesterol that self-associate into bilayers encapsulating an aqueous interior. The word liposome does not in itself denote any size characteristics as is frequently presumed and therefore is not an alternative to a nanosome. Their unique property that within the alternating aqueous or lipid compartments of liposomes, water or lipid-soluble substances respectively can be entrapped (Bangham, 1968) makes liposomes most desirable for drug delivery as they can carry both hydrophobic and hydrophilic molecules. By making liposomes in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane) they can be (indiscriminately) delivered past the lipid bilayer.

Another interesting property of liposomes are their natural ability to target cancer. Unlike healthy human blood vessels having tight junctions to stop any large particle in the blood from leaking out of the vessel, tumour vessels do not contain the same level of seal between cells and are diagnostically *leaky*. Liposomes of certain sizes, typically less than 400nm, can rapidly enter tumour sites from the blood, but are kept in the bloodstream by the endothelial wall in healthy tissue vasculature. Anti-cancer drugs such as Doxorubicin (Doxil®) (Haran *et al.,* 1993 and Gabizon *et al.,* 2003), and Daunorubicin (Daunoxome®) (Piccaluga *et al.,* 2002) are currently being marketed in liposome delivery systems. While vincristine (Waterhouse *et al.,* 2005) (Onco TCS™) awaits FDA approval (Gelmon *et al.,* 1999). However, there have been major drawbacks to the use of liposomes for targeted drug delivery, most notably, poor control over release of the drug from the liposome (i.e. the potential for leakage of the drug into the blood), low encapsulation efficiency, manufacturability at the industrial scale and poor stability during storage (Hans and Lowman, 2002 and Soppimath *et al.,* 2001).

The ultimate goals of nanomedicine is to create medically useful nanodevices that can function inside the body (Arayne and Sultana, 2006). When it comes to oral drug delivery, the dose has to navigate pH gradients, protective membranes, pass through hydrophobic and hydrophilic

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regions, continue its journey through opposing solubility and absorption regimes, survive a variety of metabolic and transport proteins that seek to modify and excrete the foreign compound, and then traverse the plasma or lymphatic systems to the site of action. It must act only on the target organ, and not in other places where it could cause harm to the organism. All of this is accomplished under constraints including limited gastrointestinal transit time, presence or absence of a huge variety of other chemical compounds that comprise our food, drink, and other medications, and elimination half-life. It is rather miraculous that the little pills can cure anything at all, yet they do, often to a remarkable extent (Edgar, 2007). Such is the science of drug delivery; transporting medications where they are supposed to go and most revolutionary research has been done in the last couple of years encompasing field of nanotechnology and nanofabrication to improve drug delivery which includes to development of targeted delivery in which the drug is only active in the target area of the body (for example, in cancerous tissues) and sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation.

Magic bullet, a concept given by Ehrlich (Ehrlich, 1960) brought targeted delivery to the fore front, but "magic bullet" continues to be a challenge to implement practically. Among other challenges, finding a means of carrying the drug in a stable form to its target sites while avoiding the immunogenic and nonspecific interactions that efficiently clear foreign material from the body proved to be more difficult. Since the efficiency of drug delivery is directly related to particle size nanoparticle formulations can enhance bioavailability, improve timed/controlled release of drugs, and enable more precise targeting to the level of direct intracellular delivery (Allemann *et al.,* 2005).

Nanoparticles are potentially useful as carriers of active drugs and, when coupled with targeting ligands, may fulfill many attributes of a 'magic bullet'. Prof. Speiser in the late 1960s developed the first nanoparticles for drug delivery purposes and for vaccines. Infections like tetanus and diphtheria require multiple injections to build up antibody levels in the body that are sufficient for protection. The objective was sustained drug release from nanocapsules that would be able to circulate in the blood after intravenous injection. In order to test the feasibility of a sustained release from such capsules, Speiser first focussed on the development of nanoparticles for vaccination purposes, with the hope that due to the sustained release properties of nanocapsules a constant immune stimulation would be achieved, and only one injection would suffice to achieve the necessary antibody response (Kreuter, 2007).

Helmut Kopf process (Kopf, 1975, Kopf *et al.,* 1976 and 1977) was Speiser's student who bound norephedrine HCl and 1-[4-(2-biphenyloxy)-butyl]-1-ethylpiperidinium bromide into the acrylamide nanoparticles and also performed a thorough chemical and physicochemical investigation of the nanoparticles and of the production process.

Oppenheim joined Speiser's group in 1974 and developed gelatin and albumin nanoparticles by a process (Marty, 1977 and Marty *et al.,* 1978) that was improved 20 years later (Coester *et al.,* 2000) and used for the transport across cell membranes of oligonucleotides (Wartlick *et al.,*2004a and 2004b), peptide nucleic acids (PNAs) (Langer *et al.,* 2000), and even genes (Rhaese *et al.,* 2003). Cell targeting using these nanoparticles was improved by the covalent attachment of antibodies (Wartlick *et al.,* 2004a and 2004b and Balthasar *et al.,* 2005), and the transport of drugs across the BBB was achieved by the covalent binding of apolipoprotein E to the surface of human serum albumin nanoparticles produced using the method of Oppenheim (Michaelis *et al.,* 2006).

Some more nanoparticles were developed (Zolle *et al.,* 1973 and Scheffel *et al.,* 1973), Kramer (Kramer, 1974) introduced these nanoparticles for drug delivery purposes and bound mercaptopurine to them. Later the same process was used to produce magnetic nanoparticles that could be targeted by an external magnetic field [Widder, *et al.,* 1978, 1979a and 1979b). However, it is much more difficult to focus the particles by a magnetic field deeper inside the body. For this reason, magnetic targeting has not so-far found broad application.

There still is some confusion over the nomenclature of nanoparticles: the terms "nanoparticles", "nanocapsules", "nanospheres", "microcapsules", "microspheres", "colloidal carriers", and "latices" all are used interchangeably for the same thing: particles in the nanometer size range for the delivery of drugs or other biologically active materials. This makes a literature search difficult, because expressions like microcapsules and microspheres also include larger particles. Terms like "latices", on the other hand, are mainly used for non-pharmaceutical applications. For this reason, some 25 years ago, attempts were made to introduce a comprehensive definition (Kreuter, 2007). For pharmaceutical purposes: "Nanoparticles are solid colloidal particles ranging in size from 10 to 1000 nm (1 μm), they consist of macromolecular materials in which the active principle (drug or biologically active material) is dissolved, entrapped, encapsulated and/or to which the active principle is adsorbed or attached" (Kreuter, 2004).

There are many ways in which nanoparticles are better than liposomes; they have better physical stability because of their solid structure and can be designed, using a wide range of materials, to control release of encapsulated drug, drugs may be bound to nanoparticles in various forms, such as a solid solution, dispersed or adsorbed on the surface or chemically attached, the surface of nanoparticles can be modified to prolong their blood circulation and coated or attached with targeting ligands to achieve site-specific drug delivery, they can deliver a relatively concentrated drug dose compared to the prodrug or drugvector approach, they may mask the circulation and BBB limiting characteristics of the drug molecules to enhance brain uptake, reducing side effects, they may offer protection to the drug molecules during transportation in the circulation, and may provide sustained release of drug to prolong the pharmacological action of drug molecules, and more (Chen Y *et al.,* 2004). These astounding features of nanoparticles can be further exploited to improve drug delivery; surface properties are the key factors, which determine the *in vivo* fate of such carriers (Asif *et al.*,2006). There are innumerable examples where nanoparticles are applied in drug delivery and in almost all therapeutic conditions be it cancers and tumors (Wong *et al.,* 2007, Murthy and Reddy, 2007, Kommareddy *et al.,* 2007, Betancourt *et al.,* 2007 and Leuschner and Kumar 2005) diabetes (Devarajan and Sonavane, 2007), restenosis (Labhasetwar *et al.,* 1997), ocular diseases (Chu *et al.,* 2002) and protiens and peptides can now be formulated as oral dosage (Sakuma *et al.,* 1997, Rieux *et al.,* 2006 and Lee *et al.,* 2007) even insulin can be administered orally (Cui *et al.,* 2006 and Değim *et al.,* 2006) and many more (Roney *et al.,* 2005 and Muro *et al.,* 2006).

Nanoparticles in cardiovascular drug delivery

Cardiovascular disease (CVD), principally heart disease and stroke, are the leading cause of death and morbidity in industrialized nations and are becoming an urgent health problem for all nations due to the unstoppable trend of an ageing and obese population. Due to the rapid development of nanotechnology in recent years, management, and therapy of cardiovascular diseases has improved a great deal (Kong *et al.,* 2006).

No single technology offers a solution for all problems. However, rapid evolution and discoveries in bioengineering have created many new nanotools to address these challenges. Pharmaceutical nanoparticles have emerged as multifaceted systems capable of identifying and characterizing early disease before the gross anatomical manifestations (Lanza *et al.,* 2006). With the advent of nanotechnology, the potential for nanoparticles cardiovascular disease is infinite, with novel new applications constantly being explored (Gwinn and Vallyathan, 2006).

Nanotechnology offers four areas, in which cardiovascular diseases can be better combated with immediate impact, *Targeted therapeutics*: delivering drugs where they are needed. *Tissue engineering*: building new tissues to replace defective valves, damaged heart muscle, clogged blood vessels, and so forth, *Molecular imaging*: using "smart" imaging agents that identify disease more specifically and *Biosensors and diagnostics*: improved diagnostic devices for the lab, and implantable sensors to detect problems inside the body.

Cornering targeted therapeutics, in the field of cardiology, nanoparticles have a further advantage over larger microparticles, because they are better suited for intravenous (IV) delivery. The smallest capillaries in the body are 5 to 6 μm in diameter. The size of particles being distributed into the bloodstream must be significantly smaller than 5 μm, without forming aggregates, to ensure that the particles do not form an embolism (Yoon, 2004). Recent research has focused on the design of liposome and polymer-based nanocarriers for drug targeting to endothelial cells. Functions of nanocarriers include: 1) optimization of a drug's pharmacokinetics in the bloodstream and protection of drugs against inactivation and premature activity en route to the target; 2) fine control of drug-release kinetics; 3) providing a template for multivalent affinity binding sites enhancing effectiveness of anchoring on the target cells; and 4) modulation of subcellular delivery of drugs (Bi-Sen *et al.,* 2006).

The local drug delivered approach has an important advantage of decreasing the total drug burden while greatly augmenting the drug concentration in the target site of the artery (Fishbein *et al.,* 2001). Moreover, various polymer-based drug delivery systems allow for a prolonged drug presence in the delivery site, thus making possible the use of unstable drugs that are rapidly degraded when administered in the unprotected free form (Chorny *et al.,* 2000, Fishbein *et al.,* 2000a and 2000b and Golomb *et al.,* 1996). Figure 1 illustrates the structural design of some currently available nanocarriers [Bi-Sen *et al.,* 2006).

Phospholipid-based liposomes arguably represent the most extensively studied drug vehicles (Moghimi and Szebeni, 2003 and Mainardes and Silva, 2004). Phospholipids forming bilayers in aqueous media provide capsular vehicles, the internal aqueous space of which can be used for delivery of hydrophilic drugs while the lipid bilayer can be loaded with small hydrophobic drugs. This clearance can be markedly delayed by the grafting of synthetic hydrophilic polymers such as poly(ethylene glycol) (PEG) onto the surface of the vehicles. Such "stealth" DDS have prolonged pharmacokinetics and lesser side effects of activation of host defense (immune response, cytokine release, complement activation)

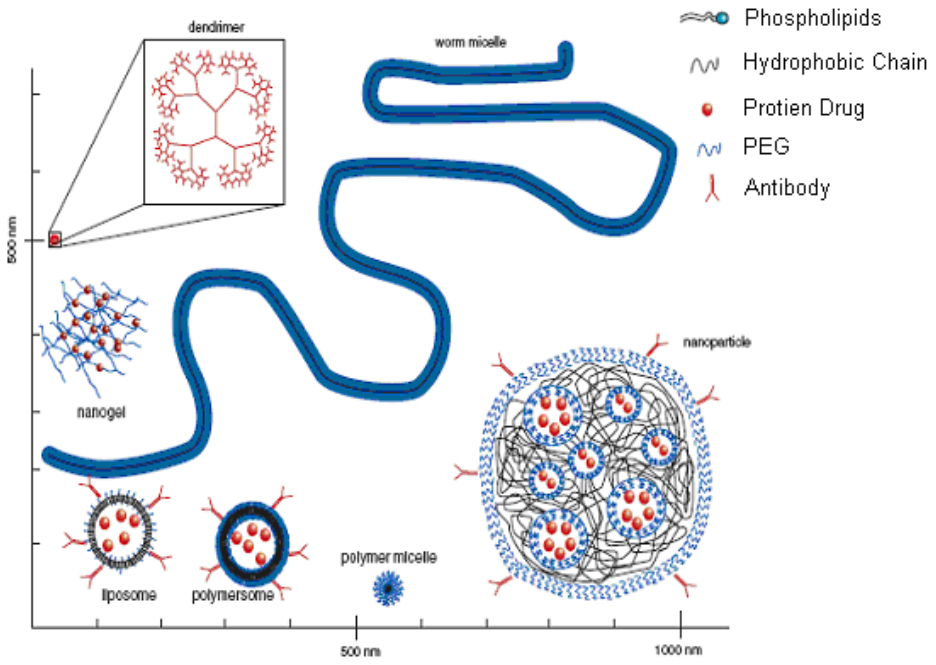

Fig. 1: Nanocarriers for vascular drug delivery.

(Moghimi and Szebeni, 2003). Internal aqueous domain of polymersomes can be loaded by hydrophilic drugs with sizes up to 500 kD, although the encapsulation efficiency decreases with increasing molecular mass (Ahmed and Discher, 2004) Liposomes only 100 nanometers across or 1/100 the size of a single cell, are used as drug carriers that could be injected to directly target damaged areas of the arteries in intravascular disease (Marci, 2002). Previously tested conditions for drug encapsulation during the nanoparticle formulation process led to the inactivation of heat-sensitive drugs and enzymes, but new methodologies have created polymer nanoparticles encapsulated with significant amounts of active enzymes (e.g., 15-20% yield encapsulation of catalase), which are protected from the proteolytic environment by the polymer shell (Dziubla *et al.,* 2005).

Dendrimers (Lee *et al.*, 2005) the smallest of nanocarriers – a few nanometers in diameter and possess multiple end groups suitable for high extent of coupling targeting or active agents, represent polymer chains branching at regular bifurcating intervals, providing rapid expansion in the number of end groups with increasing molecular mass. Dendrimers are typically very uniform in size, and can achieve very high molecular masses beyond 1,000 kD. Because of their highly branched structure, they tend to adopt a spherical geometry that provides dendrimers with a very low inherent viscosity and very high surface-to-volume ratio, as compared to linear polymers of the same molecular weight. Nanogels (Vinogradov *et al.,* 2002), based on cross-linked hydrophilic materials forming insoluble gels that swell in water, because of the hydrophilic nature of the inner volume and structural components of nanogels, loaded drugs remain in a native conformation; nonetheless, uncontrolled aggregation is a technical challenge for formulation of injectible nanogels. The main requirement for any cardiovascular nanocarrier is biocompatibility, which means they can be injected intravenously without toxicity and overt side-effects including activation of leukocytes, platelets, complement, coagulation, and kinins (Anderson and Langone, 1999). Poor drug residence in the arterial wall hinders clinical implementation of local drug delivery strategies for the treatment of restenosis, but now resolved by new type of nanoparticles (Fishbein *et al.,* 2001).

Cardiovascular molecular imaging is another area where nanotechnology is serving, using several "smart" imaging nanoparticle agents such i.e., dendrimers, liposomes, polymer delivery molecules, cantilevers, nanoscaffolds, nanofibers as potential candidates in cardiac visualization. The term molecular imaging can be broadly defined as the in vivo characterization and measurement of biologic processes at the cellular and molecular level. In contradistinction to "classical" diagnostic imaging, it sets forth to probe the molecular abnormalities that are the basis of disease rather than to image the end effects of these molecular alterations. Most important, however, the activatable near-infrared fluorescence approach holds promise for imaging of a number of endogenous proteases

involved in cardiovascular diseases in humans (Ralph and Umar, 2001). These emerging techniques provide opportunity of tracking functional and structural changes in myocardium and heart tissue (Sharma, 2006). The targeting system described as a molecular zip code, for example, an antibody that binds to specific cells in the body, allows for the precise delivery of the load in the body (Buxton, 2004). A molecular imaging agent (or imaging reporter) typically consists of two components: 1) a detection moiety, such as a radioisotope, magnetic compound, fluorochrome, or sonic enhancer; and 2) a molecule-specific or cell-specific affinity ligand, such as an antibody, peptide, or small molecule (e.g., derived from diversity oriented synthesis). Clinically useful molecular imaging agents report on specific biological processes, generate a strong signal (ideally when recognized by its target), possess favorable pharmacokinetics and biodistribution, and exhibit an excellent safety profile. For these reasons not all biomarkers associated with atherosclerosis (Ridker *et al.*,2004) furnish promising imaging targets.

Imaging technologies allow for visualization of the body based on different forms of energy-tissue interactions. They can be used to image 3-D cardiovascular structures, assess biophysical parameters such as ventricular function, stress, and strain, and monitor physiological events such as changes in vascular blood flow and myocardial perfusion. Although some imaging methods [magnetic resonance imaging (MRI), ultrasound (US)] rely mainly on energy/tissue interactions, others [fluorescence reflectance imaging (FRI), fluorescence mediated tomography (FMT), bioluminescence imaging (BLI), single photon emission computed tomography (SPECT), positron emission tomography (PET)] require the administration of imaging agents to generate a physical signal. Common to all of these methods is the ability to transform a detected signal into an image. Nuclear imaging agents have been developed for a larger number of cardiovascular processes and targets including apoptosis and angiogenesis, viability, atherosclerosis, and thrombosis (Blankenberg and Strauss, 2002, Khaw, 1999, Mari and Strauss, 2002 and Knight, 2001).

Functional imaging is opening new frontiers in cardiovascular science and molecular imaging allows investigators to identify and track cell migration in human subjects, as well as in model systems of disease (Richard *et al.*,2007). Using these tools are advancing not only basic knowledge about biological processes but also the further evolution of imaging technologies that will contribute to improvements in clinical medicine. Various types of nanoparticles are showing promise as imaging agents. Monocrystalline iron oxide nanoparticles**,** or MIONs, are showing promise for several MR imaging applications, both as particles and as particles with targeting molecules attached to the surface (Knauth *et al.,* 2001). When the surface of these particles is altered, they can remain in the circulation for times varying from tens of minutes to hours. A rapid-clearing agent allows contrast-enhanced MR imaging of the arteries and veins (Arbab *et al.*,2001). In cardiovascular imaging, Doppler processing of echo data provides real-time measurements of blood flow. Due to its high temporal resolution and its inclusion, it is a powerful technology for noninvasive assessment of heart and vascular function. MR imaging and CT also offer many opportunities to measure cardiovascular functions that were previously accessible only with invasive angiographic procedures.

Recent investigations (Flacke *et al.,*2001) have resulted in the development of a fibrin targeted, paramagnetic, nanoparticulate MR contrast agent that allows enhanced sensitive detection and quantification of occult microthrombi within the intimal surface of atherosclerotic vessels. This agent is a ligand-directed, lipid-encapsulated, liquid perfluorocarbon nanoparticles (250-nm diameter); it has a prolonged systemic half-life and can carry high gadolinium-based–agent payloads. Other investigators (Zhao *et al.*,2001) have also introduced a site-targeted MR contrast agent by conjugating a C2-glutathione S-transferase fusion protein to superparamagnetic iron oxide nanoparticles, which allows noninvasive detection of apoptosis at MR imaging. The above-mentioned site-specific US liposomes and microbubbles and paramagnetic nanospheres can be combined with microparticle-mediated vascular gene therapy (Tiukinhoy *et al.*,2000)

Arterial thrombosis plays a critical role in acute coronary syndromes and stroke. Therefore, the ability to detect thrombus in vivo has a significant clinical implication. Fibrin deposits in old, organized thrombi have recently been confirmed by histopathologic study after thrombectomy in percutaneous coronary intervention after acute myocardial infarction (Rittersma *et al.,*2005). Therefore, a noninvasive modality with a fibrin-targeted contrast agent might be ideal for in vivo detection of all thrombi. Early-phase clinical studies (phases I and II) are currently testing the safety of EP-2104R in humans (Sirol *et al.,* 2005). Noninvasive imaging of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) with the monocrystalline magnetic nanoparticles consisting of a 3-nm core, an overall size of 38 nm, may identify early stages of inflammatory cells in human atheromata *in vivo* (Matthias *et al.,* 2006). Neovascularization is a critical component in the progression of malignant melanoma. In a competitive cell adhesion assay, incubation of $\alpha_v\beta_3$-expressing cells with targeted nanoparticles significantly inhibited binding to a vitronectin-coated surface, confirming the bioactivity of the targeted nanoparticles, lowering the limit previously reported for detecting sparse biomarkers with molecular MRI *in vivo* also to detect very small regions of

angiogenesis associated with nascent melanoma tumors, and to phenotype and stage early melanoma in a clinical setting (Anne *et al.,* 2005).

Research has also covered hypertension (Verger *et al.,*1998 and Ahlin *et al.,*2002) and hyperlipedimea, synergistic to cardiovascular diseases but still if critically observed, cardiovascular diseases are not as much benefited from nanotechnology in terms of drug delivery (other then molecular imaging) as the field of cancer, tumor and others. There are still unmet aspects of cardiovascular drug delivery that need to be worked upon and sooner the better since man has traveled from stone age to present age, the so rightfully termed as *nano age*, there should not be a field deprived of this ahead-of-its-time technology.

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