Bionanotechnologies for treatment and diagnosis of Alzheimer’s disease

Alzheimer’s disease (AD) is the most common cause of dementia and accounts for an estimated 60–80% of cases. This disease usually occurs in the very elderly, where early clinical symptoms are often marked with a decline in memory, reasoning, and planning. Affected individuals are also likely to develop seizures, hypertonicity (increased muscle movements), and incontinence (loss of normal control of the bowel or bladder). Of 5 million cases of dementia in Europe, 3 million are classified as AD, and globally more than 30 million people suffer from AD. In their 2010 report, the Alzheimer’s Association (United States) estimated that 5.3 million Americans of all ages have AD, and 96% of this figure corresponds to people aged 65 and older. The same report further states that “one in eight people aged 65 and older (13 percent) have AD” and “every 70 seconds, someone in America develops Alzheimer’s and by mid-century, someone will develop the disease every 33 seconds.” Currently, AD is the fourth leading cause of death in adults after heart disease, cancer, and stroke. Although substantial progress has been made in the molecular and cellular understanding of AD, no treatment is available to slow or stop the deterioration of brain cells in AD. Five drugs have been approved by the US Food and Drug Administration that temporarily slow worsening of AD symptoms for 6–12 months in half of the individuals who take them. These are donepezil HCl (Aricept; Pfizer Inc., New York, NY), galantamine hydrobromide (Razadyne; Ortho-McNeil Neurologics, Titusville, New Jersey), memantine HCl (Namenda; Forest Pharmaceuticals, Earth City, Missouri), rivastigmine tartrate (Exelon; Novartis, Basel, Switzerland), and tacrine HCl (Cognex; Shionogi Inc., Florham Park, New Jersey). Approximately 90 experimental therapies are in different stages of clinical evaluation. People with AD are heavy users of health care, long-term care, and hospice care, and this situation is having a significant impact on the global health care system. Indeed, the Alzheimer’s Association has estimated economic costs of US $172 billion for AD in 2010. As the number of people with AD grows, there remains an urgent need to identify early detection strategies and introduce effective therapies, so as to avert an overwhelming public health problem.

To this end, a pan-European effort comprising 19 research centers located in 13 European Union countries (Belgium, Denmark, Finland, France, Greece, Hungary, Italy, the Netherlands, Portugal, Slovakia, Spain, Sweden, and the United Kingdom), and supported by the European Commission Seventh Framework Programme, have started an ambitious and a challenging approach to combat AD. The project, Nanoparticles for Therapy and Diagnosis of Alzheimer’s Disease (http://www.nadproject.eu) began in September 2008 for a duration of 5 years and with a total budget of 14 million Euros. The NAD project is interdisciplinary in nature and integrates bionanotechnology initiatives and approaches to treat AD. It is the intention of this Special Issue of Nanomedicine: NBM to celebrate some of the findings and developments reported by this consortium during the EuroNanoForum 2011 in Budapest, Hungary (1-3 June 2011).

The NAD approach

Hallmarks of AD include accumulation of clumps of proteins called amyloid-β (Aβ) outside brain cells (also referred to as senile plaques) and accumulation of altered proteins inside the cells referred to as neurofibrillary tangles of hyperphosphorylated τ-protein. These plaques are the target of the NAD project through two parallel but interrelated nanoparticle-based initiatives. It has been suggested that alteration of peripheral or brain Aβ dynamics through peripheral sequestration of Aβ with agents that have a high binding affinity for Aβ may dramatically improve the condition of AD sufferers. This is based on recent suggestions that the brain and the blood Aβ are in equilibrium though the blood-brain barrier (BBB); this is also known as the “sink effect” (Figure 1). For instance, low-density lipoprotein (LDL) receptor-related protein 1 (LRP1), a member of the LDL receptor family, is a major receptor for transvascular Aβ transport across the BBB (Figure 1). Recombinant LRP1 clusters, however, can effectively sequester Aβ in AD plasma and in ApoE−/− mice (mice heterozygous for the sw mutation of the gene encoding the Aβ precursor protein), thus promoting Aβ efflux from the mouse brain. Other examples include gelsolin and ganglioside GM1, which can bind Aβ in the periphery, which critically alters Aβ clearance rates from the brain. Similar approaches are being taken in the NAD project, but based on design and engineering of a wide range of nanoconstructs decorated with Aβ-specific ligands for targeting of Aβ peptides in the peripheral circulation, thereby promoting their clearance by routing complexes to the hepatic and the splenic macrophages for destruction or by altering their aggregation kinetics. NAD investigators have modeled and designed a number of such entities demonstrating high affinity for Aβ at...
least in vitro. These include polymeric nanospheres such as PEGylated poly(alkyl cyanoacrylate) nanoparticles, libraries of solid lipid nanoparticles, and a selection of anionic liposomes (phosphatidic acid- and cardiolipin-incorporated vesicles) as well as curcumin- and Aβ-specific antibody-decorated vesicles.7-9 Curcumin is an established Aβ ligand capable of inhibiting Aβ oligomer formation; it can further bind to plaques in vivo. For liposomal and solid-lipid nanoparticle incorporation, different types of curcumin-phospholipid conjugates have been designed. Peripheral treatment with such multivalent nanoparticles is expected to reduce the level of Aβ in the brain by shifting Aβ equilibrium, thus reducing brain amyloidosis, and is currently under investigation.

Aβ-binding anionic liposomes have also been shown to rescue Aβ-induced toxicity in murine neuroblastoma cells. For AD treatment, however, liposomes (or other AD nanomedicines) should be able to cross the BBB. The BBB is a highly specialized gatekeeper structure localized at the interface between the blood and the cerebral tissue. It is composed of a tightly sealed monolayer of endothelial cells of cerebral blood vessels, which is regulated by tight junctions and adherens junctions.10 The BBB maintains brain homeostasis and therefore tightly regulates the entry of compounds (including nanoparticles) and cells between blood and brain. Furthermore, there is no evidence of permeabilization of the BBB in AD, but changes in the vascular system of the brain may significantly contribute to the progression of dementia.11 NAD investigators are further employing noninvasive nanoparticle-based approaches to overcome the BBB. This is based on design and surface engineering of Aβ-binding nanoparticles with ligands specific for cerebral
capillary endothelial cells with the capability of initiating transcytosis (e.g., a selection of engineered apolipoprotein E peptides). Promising results have now been obtained in several in vitro animal and human BBB models. These efforts are now providing the basis for development of multifunctional nanoparticles with triggered-release mechanisms, and for simultaneous in vivo imaging (e.g., magnetic resonance imaging and positron emission tomography) and treatment of experimental AD (a theranostic approach).

It is conceivable that future efforts with nanoparticle engineering could employ strategies that simultaneously affect multiple targets that are involved in pathogenesis of neurological disorders. For instance, in AD and AD models, the expression of the receptor for advanced glycation end products (RAGE) increases by several fold in affected cerebral vessels as well as in microglia and neurons, where RAGE can bind different forms of Aβ (Figure 1). Another interesting target is mesenchyme homeobox gene 2 (MEOX2), which regulates vascular cell differentiation and repair, and its expression in the adult brain is restricted to the vascular system. Low expression of MEOX2 gene in AD cerebral endothelium contribute to abnormal responses to angiogenic factors (e.g., vascular endothelial growth factor), inducing premature vessel regression. Also, low expression of MEOX2 gene may further promote proteosomal degradation of LRP1; this lowers the Aβ-clearing capability at the BBB level. Therefore, nanoparticle-based therapeutic approaches may include combination strategies comprising controlled drug release within the vasculature (e.g., low-molecular-weight RAGE antagonists), enhancing vascular Aβ clearance through hepatic and splenic mononuclear phagocytes, ex vivo modulation of cellular vectors capable of BBB translocation, and nucleic acid delivery for gene expression and silencing (e.g., enhancing LRP1 expression in the hepatocytes; enhancing LRP1, myocardin, and MEOX2 expression in cerebral endothelial cells while silencing RAGE) (Figure 2).

Particular attention must also be given to issues of nanoparticle safety at and off target, and in relation to multiple dosing. One important safety issue is nanoparticle-mediated
activation of the complement system, which is the most ancient component of innate human immunity.\textsuperscript{14} Complement activation can induce adverse reactions in certain individuals through anaphylatoxin generation and subsequent release of secondary mediators.\textsuperscript{14,15} The complement system is also activated in the brains of AD patients and plays a key role in AD pathogenesis.\textsuperscript{16} Therefore, brain-localized nanoparticle-mediated complement activation may exacerbate the pathology of AD. Because the complement system could play a central role in neuronanomedicine performance, a better understanding of material properties in relation to complement activation remains a pivotal area for precision engineering of therapeutic and theranostic nanoparticles for AD treatment and detection. Accordingly, AD-specific nanoparticles should undergo extensive “structure-activity” studies\textsuperscript{17,18} in relation to both target specificity and complement activation properties. These issues are also within the framework of the NAD project, where some of the engineered systems are constructed in architectures that do not activate complement to any great extent.

To date, the progress made by NAD investigators represents a significant step in advancement of state-of-the-art nanobiotechnology approaches that could potentially combat AD.\textsuperscript{2} We eagerly await future efforts and translation of these inventions and discoveries for the benefit of patients worldwide. Finally, the contributions of NAD contributors to this Special Issue is gratefully acknowledged.

S. Moein Moghimi, PhD
Associate Editor

References