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Nanotechnology in Personalized Medicine: A Promising Tool for Alzheimer's Disease Treatment

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Abstract

Background: Alzheimer's disease (AD) is a public health priority all over the world. The difficulty of fighting the disease consists mostly in the complexity of symptoms and causes, in the still poor knowledge of its mechanisms and in the existence of a latent, asymptomatic, preclinical stage. Although many drugs are continuously screened in clinical studies for the treatment of Alzheimer's disease, the unexpected lack of patient response and sometimes the important adverse effects make it a potential field of application for personalized medicine.

Objective: This perspective review proposes nanotechnology as a valuable tool for the application of personalized medicine to AD.

Methods: The aim of personalized medicine is the development of more patient-precise treatments based mostly on the knowledge of individual genetics as well as of disease progress, and of pharmacokinetics and metabolic response to available drugs. The optimization of new and more sensitive detection techniques is an important tool for obtaining the pool of data needed for prediction and understanding of patient response.

Results: Research in bionanosensors is already providing examples with high sensitivity for core and new biomarkers for AD. In therapy the functionalization of nanoparticle surface can add specificity for biological recognition or for improving the bioavailability. This would allow the administration of lower doses with less adverse effects due to the local targeting.

Conclusion: Taking into account the promising characteristics of nanoparticles as excellent candidate tools for precision medicine development, the establishment of better

standard methods for safety assessment and production scale up would be desirable for the nanomaterial fruitful employment.

Keywords: Nanoparticles, Alzheimer, precision medicine, nanotechnology, targeted medicine, biosensors, neurodegeneration.

1. INTRODUCTION

1.1. Alzheimer's Disease

Alzheimer's disease (AD) is considered at present a public health priority because of the increasing number of dementia cases worldwide and the lack of effective treatments [1, 2].

The complexity of this chronic disease originates from the high heterogeneity of pathological hallmarks and possible causes. At present, the diagnosis of the disease is based mainly on cognitive and functional analysis [3, 4].

Two main forms of AD can be defined, the Early-Onset AD (EOAD) and the Late-Onset AD (LOAD). EOAD, also referred as familial, represents only 5% of AD cases. It is associated to 230 mutations, within three genes related to the synthesis and proteolysis of amyloid precursor protein. EOAD appears at an age under 65 years old [5-7].

On the other hand, LOAD appears in older individuals and it can be related to different factors, among them genetic and epigenetic ones, but also environmental conditions. Genetically, the best-known and certain risk factor is represented by the presence of allele $\epsilon 4$, coding for an isoform of apolipoprotein E (ApoE) [8-10].

AD is characterized by the development of two major lesions that are amyloid plaques and neurofibrillary tangles.

The formation of amyloid plaques is due to the accumulation of amyloid β ($A\beta$) peptides around neurons that can occur either by their pathological overproduction or by their impaired clearance across the blood-brain barrier (BBB). $A\beta$ peptides accumulated in the brain are prone to aggregation, first into soluble pathogenic $A\beta$ oligomers and fibrils, and finally into insoluble $A\beta$ plaques. Accumulation of $A\beta$ plaques in the walls of small brain arteries produces a condition of cerebral amyloid angiopathy [11-15].

The second major lesion is the formation of intracellular neurofibrillary tangles, pathological aggregates in the form of filaments, of microtubule-associated hyperphosphorylated tau protein. Tau hyperphosphorylation leads to its dissociation from microtubules and so to important alterations of microtubule functions as component of cytoskeleton [8, 11, 12, 16, 17].

An incorrect functionality of BBB is also related to AD. In fact, alterations have been reported to occur in association with neurodegeneration, among them those involving many important carriers and receptors [18- 22]. However, it is still not clear if these alterations are cause or consequence of the disease.

Apart from the described most-known physiopathological hallmarks, AD development has been related with other possible causes and symptoms.

Among other reported evidences of the disease there are endogenous factors, as the production of reactive oxygen species (ROS). DNA oxidative damage and markers of oxidative stress have been reported to be significantly

higher in AD patients [23-25]. Neuroinflammation, produced by both environmental and genetic factors, also seems to have an important role in AD development since activated microglia and astrocytosis conditions have been reported in presence of amyloid plaques and tau tangles [26, 27].

Finally, other physiological conditions involved in AD development are metal imbalance in the brain and mitochondria structural and functional alteration [28-31]. A condition of metal accumulation could be responsible for toxicity associated to redox activity. The alteration of metal homeostasis seems to favor A β peptide accumulation in the brain [28, 32].

A schematic summary of the main pathological sources of heterogeneity in AD is reported in Fig. (1).

Fig. (1). Overview of the main challenges of AD treatment.

Treatments available for AD are mostly symptomatic. Although many drugs are continuously entering clinical phases, effective disease-modifying treatments are still lacking. For this reason, AD is considered at present a public health priority [33].

1.2. Personalized Medicine

During last years, more attention has been paid to personalized medicine due to the unexpected failure of treatments of different diseases, a lack of response in patients or increased adverse effects in a single individual.

Personalized medicine (also called precision medicine) represents an approach for patient treatment aimed to improve the efficacy of therapies by filling the gap between the expected effect and the individual response. It refers to optimally targeted and timed treatments that prevent, stop, or slow disease progression based on individual's molecular knowledge [34].

It has been observed that the concept of “one-size-fits- all” seems to fail more and more in medicine, especially in the treatment of diseases with high heterogeneity of causes and physiological symptoms. Heterogeneity, referred to both disease development pathway and patient response, is the central problem that leads to the concept of precision medicine. Different patient responses to the same therapeutic agent can depend on both genetic and environmental factors. Actually, personalized treatments in clinical practice have been applied since long time and they can be originally referred to the concept of choosing a treatment on the basis of a diagnosis of a disease in a patient. Blood typing before transfusions and the prescription of a specific antibiotic based on previously determined

sensitivity of infection in a single patient are examples of personalized medicine already used in clinical practice.

Personalized medicine is based on the knowledge of molecular mechanisms in individuals through pharmacogenetics/pharmacogenomics analyses. In this sense, the genomic approach for the identification of DNA sequence alterations, strongly prompted by the obtaining of the complete human sequence of genes by the Human Genome Project (HGP), represents one of the most powerful tools for personalized medicine. Also, transcriptomics, proteomics and metabolomics can be used both to predict disease development and to better manage it in single patients [35-38].

The knowledge of patient clinical data, his genotyping and protein expression, especially drug-metabolizing enzymes, is an important support for the precise design of the most effective therapy with the lowest adverse effects [36, 39].

In many cases the use of precision medicine is thought as too expensive to be used at large scale, mostly due to the complexity of the analytical techniques employed to obtain the necessary knowledge of the patient genomic pattern. However, the prediction of drug metabolism would allow the formulation of a more patient-specific and effective treatment regime, which would ultimately decrease the total cost for medical treatment. The same happens when preventive measures can be taken, thanks to the identification of risk factors of developing the disease after an inherited predisposition [39].

AD, with its complexity and lack of effective treatments, represents a very interesting field for precision medicine application. The variability in genetic

predisposition and symptoms lead to the mistaken practice of treating patients with different profiles as a single group and submitting them to the same clinical treatments.

Inter-individual differences in the metabolism of a drug lead to ineffectiveness toward a particular class of drug in 70% of Alzheimer's patients [40]. Also, differences in the rate of clinical progression should be taken into account for the selection of appropriate therapy [7].

The main key points of personalized medicine, that are risk assessment, early diagnosis and personalized clinical treatment, could be successfully applied to AD.

In Fig. (2) the general approach of personalized medicine is schematically represented.

Fig. (2). Schematic summary of personalized medicine approach.

A comprehensive risk assessment from both genetic and environmental points of view is fundamental to establish a monitoring protocol and so to start a treatment in the very early stage of the disease. In fact, the development of AD, both in LOAD and EOAD, consists of three main stages that are latency (or preclinical stage), in which no symptoms appear but structural and functional changes in the brain are already occurring, prodromal stage, characterized by mild symptoms and finally the dementia stage, when symptoms of the disease become fully manifest [41-43].

Genetic risk assessment, as well as the detection of the disease during the latency stage, represents the most important challenges in the treatment of AD. Multi-study consortia recently conducted large-scale genome-wide association studies, leading to the determination of a list of candidate genes for further investigation about their role as risk or protection factors in LOAD [44, 45].

Personalized medicine approach involves a preliminary family history and genetic analysis of the patient (especially for EOAD) and allows advising the patient about modifiable environmental risk factors for AD to pay attention to, like obesity, hypercholesterolemia, sedentary lifestyle and smoking [43]. Recently ApoE ϵ 4 allele on chromosome 19 was demonstrated to be a LOAD susceptibility gene. It has been advised that patients presenting this isoform of A β transporter apolipoprotein E should avoid participation in contact sports or other activities that may provoke brain injury, which is considered a condition highly favoring the development of the disease in the presence of a genetic predisposition [43, 44, 46].

The use of genetic markers can be of great importance also for the design of clinical trials. ApoE ϵ 4 allele has been used as biomarker for the selection of patients for two risk-assessment trials (Alzheimer's Prevention Initiative and TOMORROW) on no symptomatic individuals [45].

Another key point of personalized medicine that can be applied to AD is represented by the use of modern analysis tools and diagnostic techniques (including neuroimaging and biomarkers analysis) especially for early diagnosis during the preclinical stage.

Techniques for the detection of latent pathological AD condition have been improved significantly, especially for what concerns brain imaging technologies. High-quality structural magnetic resonance imaging (MRI) is used at present to identify structural lesions and neuroanatomical signatures, photon emission tomography (PET) and the single photon emission computed tomography (SPECT) are used to determine the presence of specific metabolic/perfusion signatures. At the same time, researchers are constantly improving the sensitivity of biomarkers analysis in cerebrospinal fluid (CSF) and blood [7, 46].

Also, several programs and initiatives are working for the continuous improvement of early diagnosis of AD. ADNI (Alzheimer's Disease Neuroimaging Initiative) is a program based on a private-public partnership. Its objective is to collect data for the assessment of clinical parameters for the best design of clinical trials. Thanks to the use of the previously mentioned techniques of analysis, ADNI is collecting an important amount of data that will allow the

understanding of mechanisms occurring during latency and prodromal phase of the disease [45].

The total concentrations of tau (total tau), the concentration of tau phosphorylated at specific threonine and/or serine residues and the concentration of A β peptide 1-42 are altered in the cerebrospinal fluid of Alzheimer's patients and their potential use as biomarkers of the disease is especially important because the alteration of their concentrations can be related to mild cognitive impairment stage [47]. They allow a good degree of differentiation from other neurodegenerative diseases and have been included in clinical diagnostic studies in some countries [48]. However, a high variability in the measured concentrations has been reported, mostly due to variation in the analyzed populations (possibly different stages of AD), pre-analytical procedures (from lumbar puncture to sample handling) and assay-related factors of variability [49, 50].

On the contrary, PET neuroimaging for the detection of amyloid β lesions is considered a very reliable technique with high reproducibility of results and interpretations between different clinical centers [51, 52]. Unfortunately, the utility of this technique for certain diagnosis of preclinical AD remains unclear. A high percentage of asymptomatic individuals showed the presence of A β plaques in their brains, leading or not to future development of the disease. Risk assessment in this case seems to be possible only in the case of negative results [53]. A promising application of this technique is PET with fluorodeoxyglucose, a method used to measure cerebral metabolic activity through metabolic rate of glucose [43]. By this technique, it has been demonstrated that AD is characterized by local hypometabolism. A decrease in glucose transporters, both in the blood

brain barrier and in neurons, responsible for impairment in glucose uptake/metabolism in the brain, has been related to AD pathological condition [22].

MRI is another useful technique for AD diagnosis that allows brain structural analysis for the eventual detection of atrophy, cortical thickness and volumes to be related with cognitive impairments. A progressive decrease in volume of hippocampus with decreasing cognitive performance, together with a general decrease in cortical thickness were observed by MRI in AD patients. MRI usefulness in the diagnosis of preclinical stage of the disease has also been proved. Interestingly, atrophy in hippocampus, amygdala, and entorhinal cortex as well as a decrease in the volume of medial temporal lobes, posterior cingulate/precuneus, and orbitofrontal cortex that can be observed by MRI in cognitively intact individuals, have been found to be related to later cognitive decline [54-56].

Studying genotype of patients and healthy individuals with risk of developing the disease, a huge amount of data can be collected. Many initiatives have been developed during last years for the storage, analysis and availability of such data. With respect to AD in particular, some databases, restricted to clinically relevant aspects of the disease, have been developed with the aim of making information available for the selection of best therapeutic regime in single individuals. The Alzheimer's Disease Neuroimaging Initiative, the National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site and the National Alzheimer's Coordinating Centers database are some of them [39].

Intervention and treatments should be selected on the basis of the previously defined individual profile and pathological route of the disease [7, 42].

Phelix and co-workers used a computational biology model of 30 metabolic and transport pathways to simulate oral dosing of pioglitazone that is currently in clinical trials to delay onset of AD. The aim of the work is to determine the effective dosing for individual patients by simulating the response to a drug of a single patient before its administration [57].

From the point of view of personalized medicine the aforementioned unexpected failures in clinical trials could be addressed to heterogeneity of both drug metabolism and disease pathological route, as well as to the late intervention with respect to the course of the disease. Recently, more careful procedures for the selection of participants in clinical trials are being performed on the basis of genetic information and of the evaluation of the disease stage by PET or biomarkers analysis.

An example of genetic profile-related drug efficacy study is the very recent work reported by Lu and coworkers about Donepezil (Aricept®, Pfizer) (DNP). This acetylcholinesterase inhibitor was administered in Chinese patients presenting or not ApoE ϵ 3 allele to evaluate the dependence and predictability of patients' response depending on the presence of this isoform. In this case the effect of DNP was significantly higher in subjects without the ApoE ϵ 3 allele [58].

Another promising field for the study of genomic-related response to treatments is represented by the use of human induced pluripotent stem cells (iPSCs) from blood or fibroblast samples to create models called “dementia-on-

a-chip” to assess diagnostics and treatments. Patient-specific iPSCs facilitate drug screening and toxicity testing and their differentiated progenies are useful for understanding mechanisms of the diseases [45, 59].

In the last years, the key points of precision medicine have been incorporated in some innovative clinical trials based on the selection of patients’ subgroups with genetic risk for AD or sharing defined characteristics of the disease pathway.

Among them, A4 trial (Anti-Amyloid Treatment in Asymptomatic Alzheimer’s Disease) is the first prevention trial in clinically normal older individuals with an assessed risk for AD [60]. The aim of the test was to apply an amyloid-modifying treatment (Solanezumab, a humanized monoclonal antibody targeting the midpeptide domain of amyloid- β) to avoid the irreversible development/progression of the disease. Other clinical trials under testing based on precision medicine principles are the Alzheimer’s Prevention Initiative and the Dominantly Inherited Alzheimer Network Trial [61, 62].

2. NANOTECHNOLOGY FOR ALZHEIMER’S DISEASE

Nanotechnology aims at the design, fabrication and application of nanomaterials, i.e. materials with at least one dimension in the range of 1-100 nm. At this size, materials often present properties different from the bulk, such as superparamagnetism or surface plasmon resonance, that are of great interest in medicine. Furthermore, nanomaterials, in particular nanoparticles, are in the same size range as proteins and nucleic acids, placing them in a good position for interacting with those biomolecules, and in consequence, with cells. Additionally,

nanometric size is related to a large surface-volume ratio, providing advantages in applications of biological recognition and more specifically in sensing. These materials have been broadly tested for medical applications.

During the last years, nanomaterials applications have also been investigated in the field of precision medicine [63]. Herein we will discuss some reported examples of nanomaterials that could make closer the personalized medicine for AD. The classification of the examples is related to the main key points of personalized medicine: detection (risk assessment and early diagnosis), therapy and theranosis (both to personalized clinical treatment).

2.1.Detection

The core biomarkers currently used have proved extremely useful for enrichment of subjects in clinical trials but some issues such as cost and invasiveness, make advisable to develop new biomarkers [64, 65]. New biomarkers should imply minimally invasive sampling and require an economical analytical technique, optimized for sensitivity so that they could be used for a preliminary screening to reduce the number of subjects to be evaluated by a more costly/invasive amyloid test (PET imaging or biomarkers from CSF) [65]. Wishfully new biomarkers will help in staging and potentially tracking AD progress providing a good lead towards personalized medicine. Available biomarker quantification methods are not extended at point of care yet due to the need for sophisticated equipment, high expertize, complicated operations, and the challenge of very low detection limits [65]. In this mark, nanotechnology could enable for improved sensitivity, derived in first place from an effective

functionalization favored by a high surface area, and in second place from signal amplification strategies [66]. Additionally, nanotechnology allows the miniaturization of the sensor and the use of label free detection techniques, such as surface plasmon resonance (SPR) and electrochemical sensing [67, 68].

Among potential novel biomarkers soluble oligomers of A β (A β Os) have attracted a great attention in recent years as they might interact with cells membranes, specifically affecting synapses and disturbing synaptic signaling pathways, possibly leading to other AD lesions such as tau hyperphosphorylation and neuritic dystrophy [64]. However, A β oligomers are heterogeneous and present in CSF only at very low levels making difficult a reliable quantification. Several studies found higher A β oligomer levels in CSF of AD patients although there is not yet a general agreement with the available analytical techniques [64]. Several strategies have been developed from the nanotechnology to quantify these A β oligomers [68-77].

A very sensitive electrochemical biosensor, specific for A β oligomers in the presence of A β monomers and fibrils, has been reported by Xia et al. [68]. For detection, the sample was mixed with the recognition element, a A β Os-specific binding peptide functionalized with an adamantyl group (Ad-PrP(95–110)). A solution of silver nanoparticles was then added to the Ad- PrP(95–110)/sample mixture. The resulting mixture was then incubated with a beta-cyclodextrine covered electrode. The electrochemical signal was collected using linear sweep voltammetry (LSV) in a 1 M KCl solution. In the absence of oligomers, Ad-PrP(95-110) promoted the aggregation of silver nanoparticles bound to the electrode through the beta-cyclodextrin-Ad- PrP(95-110) sequence, resulting in a high signal. However, in the presence of oligomers Ad-PrP(95-100) interacted

preferentially with them and less nanoparticles remained attached to the electrode resulting in a diminished signal. With this configuration, the electrochemical signal decreased with an increase in the concentration of A β Os in the range of 20 pM to 100 nM. The limit of detection was as low as 8 pM, without any modification of the nanoparticles.

Total tau, tau phosphorylated and A β peptide in CSF are the core biomarkers to date [64-65, 69, 78-81] but less attention has been paid to tau related biomarkers compared to A β from the point of view of nanotechnology.

Demeritte et al. have recently shown a multifunctional hybrid graphene oxide based nanopatform for the label-free identification of A β and tau protein in the femto gram order of magnitude using surface enhanced Raman spectroscopy (SERS) [82]. Graphene oxide (GO) layers were functionalized with nanoparticles with a magnetic core and a gold plasmonic shell. Anti-A β and anti-tau antibodies were used as recognition elements and linked to the GO layer with a poly(ethylene glycol) (PEG) spacer. In this design, the magnetic core allowed for the sample enrichment under a magnetic field while the combination of core-shell plasmonic nanoparticles and graphene oxide enhanced the SERS efficiency. Altogether accounted for identification of β -amyloid and tau protein after magnetic separation even at 0.100 pg/mL, while the reported limit of detection in the enzyme-linked immunosorbent assay (ELISA) is 312 pg/mL for A β and 150 pg/mL for tau protein [82]. The system was successfully tested in whole rabbit blood samples with 100 ng/mL of each A β and tau protein, although those concentrations were also feasible by ELISA, which was used as control.

ApoE is a genetic marker that is already being used for the selection of patients for clinical trials. Wu et al. developed a ratiometric sensor for mutated ApoE based on graphene and mesoporous silica hybrid nanomaterials (GSHs) [67]. The GSHs were firstly prepared via a soft template-assisted reducing process with mesoporous silica homogenously coated on the surface of graphene sheets. Mesoporous silica was loaded with methylene blue (MB), an electro-active molecule, and its surface was also functionalized with ferrocene carboxylic acid (Fc) so that an internal reference was available. An assistant probe DNA linked to Fc–GSHs formed a partially complemented DNA duplex with a longer probe DNA strand. This DNA duplex prevented the electroactive MB molecules from escaping (maximal signal). If the target DNA was present, which fully complements with the probe DNA, it competitively hybridized with the longer probe DNA, opening the nanopores, releasing the MB molecules, and leading to an attenuation of DPV current. On the contrary, the internal control Fc showed no fluctuation. This signal-on strategy allows for a very low limit of detection (about 10 fM) with a concentration range of work from 10^{-8} M to 10^{-14} M. The authors claimed that the internal reference allowed for higher reproducibility, reliability and accuracy, issues that remain challenging with the currently standard techniques [67].

Also for quantification of ApoE4, Liu et al. developed another electrochemical immunosensor [83]. In this case two strategies were followed to attain higher sensitivity. First the sensing surface area was largely increased by the use of Au fractal nanostructures on the electrode, and second the signal was amplified using an enzymatic system. Imaging techniques are a more powerful tool for the diagnosis of AD and the evaluation of its progress. Low molecular

weight compounds are currently being used clinically as tracers for imaging [84, 85]. Other imaging techniques are currently under research to be applied in AD diagnosis and monitorization, with optical imaging using NIR fluorescent tracers as the most promising [86].

From the nanotechnological perspective, most of the work has been focused on the use of iron oxide nanoparticles as MRI contrast agents, quantum dots for optical imaging and in the encapsulation of low molecular weight fluorescent A β tracers [16, 87].

2.2.Therapy

Primarily nanoparticles can be used as drug carriers with one or several of these objectives, to enhance the solubility of hydrophobic drugs, to protect unstable drugs until they reach their target, to extend their circulation time and/or to obtain a targeting of the drug based on some properties of the carrier such as size or chemical nature [88]. All these features can contribute to diminish the required dose of a drug, which can be of interest for personalized medicine in a situation of low tolerance to a drug. In the absence of any other information on the carrier, the distribution of the drug in the organism will depend on the administration route, the reticuloendothelial system (RES) of the organism and under some circumstances, on the physiological features or alterations of specific tissues.

Active targeting is a strategy to achieve a higher biodistribution of the drug in the area of interest, so that the total dose can be reduced to attain an equivalent effect and the adverse secondary effects are minimized in other not-

targeted organs. It consists in grafting the nanocarrier with a fragment or a species for active cellular uptake or able to recognize a lesion or a receptor preferentially expressed in a specific tissue or organ [89]. Targeting in AD firstly involves finding a way to overcome the BBB and secondly, directing the drug to a specific lesion or area in the central nervous system (CNS) (Fig. 3). Multivalency of nanoparticles, understood as the possibility of grafting several diverse molecules on their surface, allows them addressing these two targeting tasks for drug delivery against AD. A variety of targeting species have been used in such smart drug delivery systems addressing different receptors and more examples are reported for CNS system [89-91].

Fig. (3). Schematic representation of two kinds of targeted nanocarriers, liposomes (left) and gold nanoparticles (right) as representatives of organic and inorganic nanoparticles. Organic nanoparticles are often nanostructured with hydrophobic and hydrophilic areas allowing carrying both kinds of cargos. The surface of the nanocarriers appears decorated with functional molecules for targeting, passivation of the surface or for a therapeutic action. In the case of a treatment against an AD hallmark across the BBB, two targeting moieties may be required for which the multivalency of the nanocarrier is essential.

The therapeutic strategies explored have been specially focused on the amyloid cascade hypothesis although other aspects such neurofibrillary tangles (NFT), or oxidative stress have also been addressed (Fig. 4) [16, 90]. Herein we will present some interesting examples based on the combination of several drugs treating diverse aspects of the disease as well as on active targeting.

Fig. (4). Therapeutic strategies that have been followed using targeted nanoparticles for the treatment of Alzheimer's disease.

β-sheet breaker peptide, HKQLPFFED (shortened as H102), is a promising drug against AD but is barely transported across the BBB and can be rapidly cleared after intravenous injection in its free form [92]. Zhang et al. have used dually-targeted pegylated polylactic acid nanoparticles to successfully deliver H102 to the brain after intravenous injection [92]. Targeting was accomplished with the functionalization with two peptides, TGNYKALHPHNG (shortened as TGN) and QSHYRHISPAQV (shortened as QSH), which had been

screened by phage display for penetrating the BBB and recognizing A β 42 respectively. Thiol maleimide chemistry was used for the functionalization which was optimized to obtain the maximal targeting efficacy. Biodistribution experiments after intravenous injection in mice with coumarin-loaded dually targeted nanoparticles (TQNP) afforded a drug targeting index (DTI) of 2.05, 2.11 and 3.77 for cerebrum, cerebellum and hippocampus respectively. Tests in the AD model mice showed an increased accumulation of H102 at brain A β 1–42 which led to better fibrillization inhibition and to neuroprotective effects with dually targeted nanoparticles (TQNP/H102) compared to non-modified (NP/H102) or TGN-modified nanoparticles (TNP/H102) [92].

Interestingly nanotechnology allows the simultaneous targeted delivery of several drugs in combined therapies. Kumaraswamy et al. used pegylated liposomes to encapsulate the β -sheet breaker peptide KLVFF, in combination with curcumin to fight the cytotoxicity caused by A β peptides [93]. In this case, targeting was achieved by the functionalization of the surface with lactoferrin protein, so that the liposomes can enter BBB via receptor-mediated endocytosis. Both KLVFF and curcumin were successfully encapsulated although with a low yield for KLVFF, which was proved to intercalate between the phospholipid acyl chains of the lipid carrier. The efficacy of the formulation was tested by cell viability assays using IMR-32 neuroblastoma cells incubated with A β 1-42 peptide [93].

An upstream approach to a disease-modifying drug based on the amyloid cascade is the downregulation of β -secretase, an enzyme responsible for the production of A β peptides. Several gene delivery systems have been reported to accomplish it using nanocarriers [94- 97]. Liu et al. [97] developed a targeted

system for therapeutic gene and peptide co-delivery for the treatment of the two main hallmarks of AD: A β plaque deposition and tau-related fibril formation. The nanocarrier consisted of a polyamine functionalized with RVG peptide, which promotes BBB permeability and neurons internalization via acetylcholine receptor mediated endocytosis, and a D-peptide, which breaks tau protein oligomers. After the functionalization, the carrier presented positive charge at physiological pH, convenient to electrostatically interact with a plasmid to down regulate β -secretase. In vivo experiments showed that the active targeting strategy was successful in delivering both DNA and drug to the brain. After internalization by the cells there was dissociation of the carrier so that D-peptide could exert its action against NFT whereas the plasmid could reach the nucleus.

Iron oxide and gold nanoparticles are less effective for drug delivery as they allow a lower drug cargo [16], but they offer the advantages of enabling diagnosis techniques and localized heating (hyperthermia) under an alternating magnetic field or an electromagnetic radiation. Both kinds of nanoparticles have been used for breaking the A β fibrils associated to AD by hyperthermia [98-102]. In the case of gold nanoparticles, the surface plasmon resonance wavelength can be tuned by changing the shape and/or size of the nanoparticles. In this respect, it is highly desirable to use nanoparticles able to generate heat after being irradiated in the near infrared (NIR), a wavelength range in which living matter is more transparent. For instance Yin et al. have reported on gold nanostars functionalized with PEG for stabilization in aqueous environment and with penetratin peptide, as targeting fragment to direct the nanostars across the BBB and promote cellular internalization. A Ru (II) luminescent complex was also incorporated for labelling [101]. The system proved to be able to inhibit the formation of A β fibrils and

dissociate preexisting ones upon irradiation with NIR light ($\lambda = 808$ nm). As a result, the functionalized nanostars showed neuroprotective effect over the A β -induced cellular toxicity after irradiation in vitro. The success of using penetratin for targeting was demonstrated in vitro with transwell experiments and with biodistribution experiments in vivo.

2.3.Theranosis

Theranosis consists in the simultaneous administration of a diagnosis agent and a therapeutic drug [103]. The objective is the monitorization of the effectivity of the drug so that new decisions can be taken. In addition to the application of targeting strategies, the use of nanoparticles offers several possibilities for the design of a theranostic system [16]. First, some inorganic nanoparticles can act both as reporters in diagnosis techniques, such as MRI, and, after an external trigger, as therapeutic agents in magnetic or photothermal hyperthermia. Up to our knowledge, no system has been reported against AD so far in which the inorganic core plays both roles. Targeted gold nanoparticles have been reported to block the fibrillar growth using hyperthermia, and systems based on magnetic iron nanoparticles have been used as MRI reporters and as drug carriers but not as therapeutic agents by themselves [102, 104]. As an example, Skaat et al. described the use of γ -Fe₂O₃ nanoparticles fluorescently labelled conjugated with anti-amyloid β monoclonal antibody BAM10. The system proved to be efficient in the inhibition of A β 40 fibrillization. BAM10 provided the specific recognition of A β 40 fibrils and enabled their detection ex vivo by both MRI and fluorescence imaging [104].

In a very recent work, upconversion nanoparticles (UCNPs) functionalized with a Cu^{2+} chelator were used for imaging of $\text{A}\beta$ ($\text{A}\beta\text{-Cu}^{2+}$ conjugate) both in vivo and in vitro. The UCNPs performed as energy donor for the luminescence resonance energy transfer (LRET) while Cu^{2+} was chosen as energy acceptor, allowing the suitable use of NIR for excitation. The system ability for sequestering Cu^{2+} ions could accelerate the nontoxic structural transformation of $\text{A}\beta$ [105].

A second approach involves the use of an organic nanocarrier to deliver either a single molecule active both for diagnosis and therapy [106], or a separate species for each function [107, 108]. The group of Kandimalla developed a system based on chitosan nanocapsules for the targeted delivery to the brain of diagnosis agents and drugs after intravenous administration. Targeting was achieved with the functionalization of the nanocapsules surface with the anti-amyloid antibody IgG4.1 or its F(ab')_2 . For diagnostic purposes the nanocarrier could be radiolabeled with I^{125} for SPECT or functionalized with Magnevist for MRI. Several drugs have been loaded in this system: immunosuppressant cyclophosphamide, curcumin or dexamethasone complexed hydroxypropyl-beta-cyclodextran. These nanocarriers were effective in targeting cerebrovascular amyloid in both in vivo and in vitro studies and allowed ex vivo MRI and SPECT in mice. The nanocarrier facilitated the uptake of the drugs by cells as shown by an increased internalization of the targeted nanocarrier compared to the antibody alone [107].

3. CONCLUSION AND PERSPECTIVES

AD is a heterogeneous disease in its causes and its mechanisms. As a result, promising therapeutic drugs were discarded after clinical trials due to the lack of significant improvement or to unexpected adverse effects in some individuals. These facts made of AD suitable for the development of treatments following the approach of personalized medicine. From this point of view there is a current need for methods for early diagnosis, in the asymptomatic stage of the disease, and for preselection protocols of the individuals taking part in the clinical trials, based on the genetic predisposition and on the progress stage of the disease.

In this situation of lack of treatments and failure of clinical test of new drugs, it is urgent to develop all the tools in hand that may allow the application of personalized medicine: more sensitive, accurate and user-friendly sensors, together with timely and individually adjusted therapies.

Nanotechnology represents a potentially valuable tool in both aspects. It has already proved useful in the development of more sensitive sensors and in the near future we expect this will make new biomarkers available for consideration.

The advantage of the use of nanocarriers lies in their versatility and in the unique properties that characterize some nanomaterials. Nanoparticles offer wide possibility to be biochemically functionalized on their surface to add specificity of biological recognition, to improve the circulation time and to protect the drug to be delivered from eventual enzymatic damage. In the mark of precision medicine, such characteristics translate into lower doses to be administered, improved efficacy and less adverse effects, as a consequence of the specific targeting or improved residence time.

In particular, the application of nanotechnology-based tools for Alzheimer's disease is especially interesting since it has been found that many kind of nanocarriers can cross the BBB thus allowing the delivery to the brain of drugs that could not have been taken into consideration before [109, 110].

Unfortunately, the use of nanotechnology in medicine could also be considered at present a source of uncertainty in many terms such as toxicology issues, scale-up and costs. The opinion of many authors is that the application of nanomaterials has not been experienced enough to affirm that they are completely safe for medical use and many differences can be observed comparing materials of the same nature but different shape and size [111, 112]. Moreover, when the aim is to use a nanomaterial in a biological complex environment, many other parameters should be taken into account in order to assess the material behavior. More specifically, the effect of some kinds of nanoparticles on the fibrillogenesis is still not clear since it has been found that some of them could promote the aggregation of A β peptides.

However, taking into account the promising characteristics of nanoparticles that make them excellent candidate tools for precision medicine development, the establishment of better standard methods for safety assessment in the very next future would be desirable for nanomaterial fruitful employment.

More specifically for Alzheimer's disease treatment, the contribution of the use of nanoparticles for therapy and diagnosis could be very significant thanks to nanomaterial versatility and its special usefulness in terms of personalized medicine. Nevertheless, for a truly fruitful application, a more in-depth knowledge of disease mechanisms and genetic characterization are still needed.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare that this article content has no conflict of interest. Financial support by “Fundación BBVA”, “Diputación General de Aragón” (E93), Fondo Social Europeo (FSE; Gobierno de Aragón) and Ministerio de la Economía y Competitividad del Gobierno de España for the public funding of Proyectos I+D+i - Programa Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad (projects n. SAF2014-54763-C2-2-R and CTQ2015-66869-P) is acknowledged by all the authors. R.M.-R. thanks the Spanish Ministerio de Economía y Competitividad for his contract under the “Ramón y Cajal” subprogram (RYC-2013-12570).

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