NANOPARTICLES AGAINST ALZHEIMER’S DISEASE: PEG-PACA NANOPARTICLES ARE ABLE TO LINK THE Aβ-PEPTIDE AND INFLUENCE ITS AGGREGATION KINETIC

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INTRODUCTION:
Alzheimer’s Disease (AD) is a neurodegenerative disorder characterized by progressive loss of cognitive functions and specific pathological changes in the brain: the formation of extracellular β-amyloid (Aβ) peptide aggregates and tangles of hyperphosphorylated Tau protein inside neurons. Especially Aβ peptide aggregates are widely regarded as the main cause of neuronal cell degeneration and oligomeric forms are considered as neurotoxic. Despite all scientific efforts, at the moment, effective pharmaco-therapeutic options for prevention and treatment of this dementia are lacking. A possible solution could come from nanotechnology. Especially poly [(hexadecylcyanoacrylate)-co-poly (ethylene glycol cyanacrylate)] (PEG-PHDCA) nanoparticles (NPs), developed in our laboratories, exhibit not only high in vivo stability (e.g. in bloodstream) but also the ability to reach the CNS overpassing the Blood Brain Barrier [1]. In vitro experiments evidenced that NPs adsorb Apo E from serum and penetrate into rat brain endothelial cells by an endocytosis mechanism mediated by LDL-R [2]. Taken all together, these features make these NP promising candidates for a development of new therapeutic and early-diagnostic approaches of CNS disease, including AD. The aim of this study was to prepare stable PEG-PHDCA NPs, to study their ability to link/adsorb the Aβ peptide 1-42 and to influence its aggregation kinetic.

EXPERIMENTAL METHODS:
The ability of the PEG-PHDCA NPs to link/adsorb the Aβ 1-42 peptide was studied by Capillary Electrophoresis (CE). Briefly, the peptide (10 µM) was incubated with the NPs (20 µM) at 37°C and the solution was analyzed every 2 h by CE with a detection at 190 nm. This technique has been also used to check for the ability of our NPs to influence the aggregation kinetic of the peptide. To achieve this goal, the aggregation was promoted by centrifugation at 6000 g for 20 min [3], followed by the addition of nanoparticles. Finally the solution was analyzed by CE every 2 h in order to monitor the monomeric peptides, the oligomeric forms as well as the complexes between NPs and the peptides. The same experiments have also been performed at lower concentrations of Aβ peptide (e.g. 50 nM) approaching the physiological conditions. The interaction between the peptide and the NPs has also been studied by Confocal Microscopy. Briefly, FITC-labelled Aβ 1-42 (10, 1, and 0.05 µM) was mixed with Rhodamine B-tagged NPs [4] (20 µM), incubated at 37°C for 20 h and then analyzed by Confocal Microscopy. Surface Plasmon Resonance (SPR) was used to check the affinity between PEG-PHDCA NPs and Aβ peptide 1-42. Briefly, monomers or fibrils of Aβ 1-42 were covalently immobilized on a sensor chip. As a control, a BSA-coated surface was used. Then a suspension of NPs (0.3, 3 and 20 µM) was flowed onto the chip for 3 min. Interactions were detected by a real-time change in the surface Plasmon resonance signal. The interaction between the peptide and the NPs has also been studied by Thioflavin T assays which revealed the aggregation of the peptide.

RESULT AND DISCUSSION:
As expected, the Capillary Electrophoresis studies showed no alteration of the monomer peak in the positive control (only Aβ peptide). In contrast, a significant decrease of the monomer peak together with the appearance of an unknown peak (with increased intensity as a function of time) was observed in the presence of NPs. Capillary Electrophoresis performed during the peptide aggregation process evidenced a faster decrease of the monomer peak intensity in the presence of NPs compared with the positive control. Moreover, the peak corresponding to the oligomeric forms of Aβ increased in the control while it decreased in the presence of NPs. Again, the NPs induced the appearance of unknown peaks with increasing intensities as time goes. Interestingly, no difference was detected in presence of non-PEGylated PHDCA NPs compared to the positive control.
Surface Plasmon Resonance results clearly showed that PEGylated PHDCA NPs only (i.e. not PHDCA), interacted with Aβ peptide immobilized onto the sensor chip. The binding of PEGylated PHDCA to Aβ 1-42 was specific since it was not detected on immobilized BSA. Very similar results were also obtained from a chip coated with Aβ 1-42 fibrils. Moreover neither non-PEGylated nor PEGylated NPs interacted with the chip coated with BSA. Confocal Microscopy images showed a full colocalization of PEG-PHDCA-Rhodamine NPs and FITC-Aβ 1-42 and formation of aggregates. These objects were shown to be smaller when the peptide concentration was decreased. These images suggest the aggregation of the peptide at the surface of the NPs at high concentration. This hypothesis has been confirmed by Thioflavine T assays.

CONCLUSION:
Capillary Electrophoresis, Confocal microscopy, Surface Plasmon Resonance and Thioflavine T assays experiments clearly confirmed the ability of our NPs to bind the Aβ peptides. Moreover these results suggested a pivotal role played by the PEG chains in this interaction and some degree of specificity of this interaction. All these information allow us to anticipate a possible capture of soluble forms of the peptide by our NPs, both in the bloodstream and in the brain and a subsequent elimination from liver, spleen or microglial cells. This property could prevent or slow down the formation of Aβ toxic oligomers or aggregates under physiological conditions and open new routes in the field of AD therapy.

REFERENCES:

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