The NAD project: Nanoparticles for therapy and diagnosis of Alzheimer’s Disease

Preliminary results with nanoliposomes

Francesca Re¹, Massimo Masserini², Maria Gregori¹, Valeria Cassina¹, Matteo Stravalaci², Mario Salmona², Marco Gobbi²
¹ DIMS, University of Milano-Bicocca, 20052 Monza and ²Mario Negri Institute, 20156 Milano, ITALY

BACKGROUND: Amyloidoses are a vast group of disease defined by the presence of abnormal insoluble protein deposits in organs and/or tissues, that are also defined as protein misfolding diseases (PMDs). A protein is amyloid if, due to an alteration in its secondary structure, it takes on a particular insoluble form, called the β-pleated sheet. Production and accumulation of beta-amyloid peptide (Aβ), a 40-42 aminoacids fragment of the Amyloid Precursor Protein (APP), plays a central role in the onset and development of Alzheimer disease (AD). Aβ aggregate in the brain to form oligomers, fibrils and plaques that induce a progressive degeneration of neurons and synapses.

OBJECTIVES: One of the goals of the NAD project is to create nanoparticles (NPs) able to bind Aβ and possibly to inhibit its aggregation or to destroy the aggregates. Within the present investigation we describe preliminary results concerning the preparation of nanoliposomes (LIP) able to specifically interact with Aβ. The lipid composition of LIP that we use in this project is chosen on the base of the stability, biocompatibility, stealth properties and affinity for Aβ.

METHODS: Synthetic human beta-amyloid fragment 1-42 was used. Monomers oligomers and fibrils were prepared as described previously (Dahlgren KN et al., JBC 2002) and analysed by Atomic Force Microscopy (AFM). LIP were prepared by extrusion through polycarbonate filters with a pores of 100 nm diameter and were composed of cholesterol/sphingomyelin (1:1) and glycerophospholipids or glycosphingolipids. The interaction between LIP and Aβ in different aggregation state was assessed using i) ultracentrifugation on a density gradient to separate Aβ-bound to LIP from Aβ free, followed by ELSA assay and ii) Surface Plasmon Resonance (SPR), performed flowing LIP onto immobilized Aβ and viceversa. Moreover, the LIP effect on Aβ aggregation was evaluated by AFM.

RESULTS: Our results provide evidence that the lipid composition strongly affects the ability of LIP to bind Aβ. In particular, LIP composed of Chol/Sm/anionic phospholipids (phosphatidic acid or cardiolipin) display a much better ability to bind Aβ in comparison with Chol/Sm, Chol/Sm/zwitterionic phospholipids or Chol/Sm/sphingolipids. The ability of LIP to bind Aβ was dependent on the proportion of embedded anionic phospholipids tested, in the range 5 to 40% molar. The best performance was attained at 5% molar. LIP-embedded anionic phospholipids displayed a Kd value in the nanomolar range. Moreover, LIP containing phosphatidic acid or cardiolipin seem to inhibit the formation of fibrils from Aβ monomers.

1- CHARACTERIZATION OF Aβ AGGREGATES
Atomic Force Microscopy (AFM)

AFM analysis of oligomeric (A) and fibrillar (B) Aβ assemblies. Representative 2 x 2 µm x y, 5-nm total z-range Bar: 200 nm. Inset image: 200 x 200 nm x y, 5-nm total z-range. Samples were prepared by spotting 10 µL of 10 µM Aβ preparations on freshly cleaved mica, incubated for 5 min at RT and rinsed with deionized water and dried under a gentle steam of nitrogen.

AFM measurements were performed with a Nanowizard II (JPK Instruments, Germany) operating in Tapping Mode in air using stiff silicon cantilevers (Veeco MP1110LD, resonant frequencies 320kHz, spring constant 40N/m). Image data was acquired at 1 Hz scan rates and image resolution 512 by 512 points, with drive amplitude and contact force kept to a minimum.

2- INTERACTION BETWEEN NANOLIPOSOMES AND Aβ
Ultrasentrifugation and ELSA assay

Ultracentrifugation and ELISA assay

Quantification of the proportion of Aβ bound to LIP. LIP composed of Chol/Sm/anionic phospholipids display a better ability to bind Aβ in comparison with other lipid composition. The ability of LIP to bind Aβ was dependent on the proportion of embedded anionic phospholipids tested, in the range 5 to 40% molar.

3- DETERMINATION OF AFFINITY BETWEEN NANOLIPOSOMES AND Aβ
Surface Plasmon Resonance (SPR)

Sensorgrams (A) and Kd values (B) between LIP and Aβ are shown. LIP, with different lipid composition, were injected at a rate of 30 µL/min for 5 min at different final concentration (from 20 µM to 1 mM). At the end of injection the dissociation phase data were collected for 15 min. The assays were done at 25°C. The sensorgram (time course of the SPR signal, expressed in resonance units, RU) observed in the cell immobilizing the peptide was corrected by subtracting the response observed in the empty cell (reference cell) and usually normalized to a baseline of zero R.U. LIP-embedded anionic phospholipids displayed a Kd value in the nanomolar range.

4- NANOLIPOSOMES EFFECT ON Aβ AGGREGATION
Atomic Force Microscopy (AFM)

AFM analysis of Aβ aggregation with different nanoliposomes. Aβ (20µM) in 10mM Tris/150mM NaCl/1mM EDTA (pH 7.4) was incubated at 37°C for 2 days with (A) Chol/Sm/anionic phospholipids 20% nanoliposomes or (B) Chol/Sm nanoliposomes (6mM). Representative 10 x 10 µm x y, 5-nm total z-range AFM images are shown. Bar: 1µm.

CONCLUSIONS: The results suggest that LIP containing phosphatidic acid derivatives interact with Aβ with high affinity and inhibit the aggregation of monomers. Therefore LIP will be utilized in further experiments in vitro, using biological fluids and cellular models, and then in vivo on animal models of AD. Moreover, these liposomes may be further functionalized with other molecules with high affinity to Aβ, with ligands enhancing the nanoparticle’s passage through the blood-brain barrier (BBB) and with contrast agents for MRI or PET. The prediction is that nanoparticles will detect, disaggregate and remove brain Aβ. Besides this action, nanoliposomes will hopefully interact with blood Aβ, drawing out the excess of brain peptide by a ‘sink effect’. These results have been obtained within the frame of the FP7 EC project: NAD, Nanoparticles for therapy and diagnosis of Alzheimer’s Disease.