ApoE-nanoliposomes for transport of Aβ1-42-ligands across the blood-brain barrier: a study in a model system.

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The neurotoxic beta-amyloid peptide (Aβ), formed in anomalous amounts in Alzheimer's disease (AD), is released as monomer and then undergoes aggregation forming oligomers, fibrils and plaques in diseased brains. Aβ aggregates are considered as possible targets for therapy and/or diagnosis of AD and for this purpose we previously identify three molecules able to bind with high affinity the Aβ1-42 peptide: curcumin chemical derivative (Kd = 1-5 nM, synthetized in our laboratory), phosphatidic acid and cardiolipin (Kd = 22-60 nM). Since nanoparticles (NPs) are promising vehicles for imaging probes and therapeutic agents, we prepared nanoliposomes functionalized to target Aβ1-42 with high affinity, that is incorporating the curcumin chemical derivative or functionalized with phosphatidic acid or cardiolipin. Moreover, since the Aβ aggregates are localized in diseased brains, we functionalized these nanoliposomes with uptake-facilitating molecules in order to enhance the blood-brain-barrier (BBB) penetration of Aβ1-42 ligands: nanoliposomes were covalently coupled with monomer or tandem dimer of ApoE-derived peptides (a.a. 141-150, synthetized in our laboratory), at different surface densities, by exploiting the peptide cysteine reaction with a maleimide-group on the nanoparticle surface.

Aim/Methods. We studied the cellular uptake and the permeability of nanoliposomes and Aβ1-42 ligands by an in vitro model of BBB of cultured human brain capillary endothelial cells (hCMEC/D3), using fluorescence analysis (confocal microscopy and FACS) and radioactivity measurements.

Results/Discussion. Nanoliposomes without functionalization for BBB crossing did not show either relevant cellular uptake. Functionalization with both the peptides used mediated an efficient nanoliposomes cellular uptake, that increased with the surface peptide density; nanoliposomes carrying monomer ApoE-derived peptide were the best performing. Moreover, we studied the ability of nanoliposomes to enhance the transport of two Aβ1-42 ligands through a cell monolayer. The permeability of curcumin-derivative was enhanced after its entrapment into ApoE-nanoliposomes, in particular those functionalized with the dimer ApoE-derived peptide at high
surface density (+83% with respect to free drug, \(p<0.01\)). Nanoliposomes embedding acidic phospholipids and functionalized with ApoE peptides show higher permeability across the BBB in vitro (\(1.5-2\times10^{-5}\) cm/min with respect to non-functionalized nanoliposomes \(5\times10^{-6}\) cm/min) and the results obtained using dual-radiolabelled nanoliposomes, suggested that nanoliposomes cross intact the BBB in vitro. Finally, the bi-functionalization of nanoliposomes with acidic phospholipids and ApoE peptides scarcely affects their reported ability to bind the A\(\beta\)1-42 in vitro. These are important and promising features for the possibility to use these nanoliposomes for the targeting of A\(\beta\) in the brain districts.