THERAPEUTIC IMPACT OF APOJ/CLUSTERIN-COUPLED LIPOSOMES ON ALZHEIMER'S DISEASE EXPERIMENTAL MODELS

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1. Summary

Background: Currently, there are no effective treatments to cure Alzheimer's disease (AD) or to prevent the disease progression. Recent genetic and biochemical evidences have highlighted the role of apolipoprotein J/clusterin (ApoJ/Clu) as a protective molecule in AD, although its molecular contribution to the disease pathology has not been elucidated yet.

Objective: This proposal aims to study a new therapeutic approach for targeting β-amyloid (Aβ) in AD pathology based on the chronic treatment with lipidic particles coupled to recombinant human ApoJ (rApoJ).

Methodology: We plan to design, synthesize and characterize lipidic nanoparticles coupled to rApoJ protein, required for the following aims. We propose to test the effect of rApoJ- lipidic nanoparticles administrated intravenously in an experimental model of AD (APP23 transgenic mice). After a chronic treatment, we will evaluate the effects on the most important features associated to the disease, such as Aβ load and cerebral inflammation. Furthermore, we will investigate the molecular mechanisms related to the rApoJ-coupled lipidic nanoparticles treatment in different in vitro models based on brain cultured cells.

Expected Results: We hypothesize that the ability of ApoJ to bind and block Aβ aggregation, besides the fact that it can potentially target the brain across the blood brain barrier (BBB), will make the proposed rApoJ-lipidic nanoparticles appropriate to serve as a peripheral decoy to enhance Aβ drainage. We consider that this is a novel therapeutic strategy to prevent the cerebral inflammation and cognitive decline associated to Aβ deposition with promising consequences delaying the progression of the age-related neurodegeneration.

2. Results

1) Production of human recombinant ApoJ (rApoJ): Because our aim was to generate lipidic nanoparticles containing ApoJ, we first produced the recombinant protein from human cell cultures. Synthetic rApoJ resulted highly pure and conserved

the heterodimeric nature and the propensity to form high molecular aggregates. Next, we studied whether rApoJ was able to modulate the brain Aβ elimination across the BBB. For this purpose, we used a model based on primary cerebral endothelial cells cultured on matrigel-coated transwells and treated with fluorescently labeled Aβ40 to track its efflux across the BBB, which corresponds to trafficking from the basolateral (brain) to apical (blood) compartments. We observed that the transport of basolateral Aβ40 was enhanced when it was complexed to rApoJ. We also observed that rApoJ moderately crossed the monolayer (from blood to brain) through a mechanism involving the LDL receptor-related protein (Lipoprotein receptor-related protein, LRP) family. These results and the complete protocol for rApoJ production are described in Merino-Zamorano et al., Journal of Alzheimer's Disease 2016.

2) Scale-up production and characterization of ApoJ coupled-lipidic nanoparticles: We synthesized and characterized lipidic nanoparticles forming structures resembling rHDL formed with rApoJ (rHDL-rApoJ). These nanoparticles were prepared using the cholate dialysis method, based on the liposome disruption, followed by incubation with rApoJ and posterior cholate removal by dialysis. After the synthesis, rHDL-rApoJ nanoparticles were purified by ultracentrifugation in KBr density gradient. Purified rHDL-rApoJ nanoparticles were characterized by N-PAGE, dynamic light scattering, circular dichroism and electron transmission microscopy (TEM). The preparation of rHDL particles showed two-sized populations with discoidal shape. Functionally, rHDL-rApoJ maintained the ability to prevent the Aβ fibrillization and mediated a higher cholesterol efflux from cultured macrophages than free rApoJ protein. Furthermore, fluorescently-labeled rHDL-rApoJ nanoparticles were intravenously administrated in mice and their distribution over time was determined using an IVIS Xenogen® imager. It was confirmed that rHDL-rApoJ accumulated in the cranial region, especially in old transgenic mice presenting a high cerebral Aβ load. These results were compiled in the publication Fernandez-de Retana et al., Scientific Reports 2017.

3) Efficacy study of free rApoJ and ApoJ coupled-lipidic nanoparticles in APP23 transgenic mice: Our main objective was to study the effect of a peripheral increase of ApoJ in an experimental model of cerebral β-amyloidosis. For this purpose, fourteen-month-old APP23 transgenic mice were subjected to chronic intravenous treatment with rHDL-rApoJ nanoparticles or free rApoJ for 1 month. Aβ concentration

and distribution in brain, as well as Aβ levels in plasma and cerebrospinal fluid (*CSF*) were determined after treatments. Other features associated to AD pathology, such as neuronal loss and neuroinflammation were also evaluated. We determined that both ApoJ-based treatments prevented the Aβ accumulation in cerebral arteries, induced a decrease in total brain insoluble Aβ42 levels and a reduction in hippocampal neuronal loss. The peripheral treatment with rApoJ also induced an increase in the Aβ40 levels in CSF, whereas the concentration remained unaltered in plasma. In all endpoints studied, the lipidation of rApoJ did not enhance the protective properties of free rApoJ. The effects obtained after the chronic treatment with free rApoJ were accompanied by an enhancement of the expression of phagocytic markers in microglial cells surrounding Aβ deposits. Finally, despite the activation of this phagocytic phenotype, treatments did not induce a global neuroinflammatory status. In fact, rApoJ-based treatments were able to reduce the levels of interleukin-17 (IL17) and keratinocyte chemoattractant (KC) chemokine in the brain. These results are compiled in a manuscript submitted for publication at Alzheimer's Research & Therapy Journal (currently under review).

4) Effect of ApoA-I-Milano (ApoA-I-M) in APP23 transgenic mice: In parallel to the direct activities related to the original proposal, we studied another strategy to reduce the cerebral Aβ accumulation based, which was based on the modification of other apolipoproteins with Aβ-chaperone activity. In this regard, apolipoprotein A-I (ApoA-I), the major component of high-density lipoproteins (HDL) has an important role on cholesterol metabolism and shows protective properties against cardiovascular events. However, ApoA-I is also known to bind Aβ and to prevent its aggregation and toxicity in vitro. In our case, we selected the strategy to genetically modify ApoA-I by introducing a natural mutation (R173C), known as ApoA-I-Milano (ApoA-I-M), in order to test its effect in an AD preclinical model. It has been demonstrated that ApoA-I-M is more functionally effective than ApoA-I-wt in different experimental models of vascular damage (Ibanez et al. 2012; Elshourbagy et al 2014). Hence, we determined the effect of the chronic intravenous administration of rApoA-I-M in an experimental model of AD with cerebrovascular involvement (the APP23 transgenic mouse model). We found reduced levels of Aβ in brain vessels and parenchyma of mice that received rApoA-I-M, which were accompanied by a decrease in the neuroinflammation response. These results were compiled in the publication Fernandez-de Retana et al., Neurobiology of Aging 2017.

3. Relevance and future implications

Despite the high socioeconomic impact of AD, no effective disease-modifying therapy exists. Therefore, finding effective treatments for AD is still a major target of research. The determinant involvement of APOE genotype in this neuropathological disease highlights the fact that the regulation of lipid metabolism is crucial for Aβ accumulation and clearance. In this regard, ApoA-I and ApoJ/clusterin, which have a principal role on peripheral cholesterol metabolism being major components of HDLs, also act as natural chaperones and can be found bound to Aβ in brain and plasma. From this background, our hypothesis was that the modulation of the lipidic status through the treatment with genetic or structural modified forms of ApoA-I or ApoJ could ameliorate some of the features associated with cerebral Aβ deposition.

In this context, our study is the first to demonstrate that the intravenous administration of human recombinant ApoJ and a genetic variant of ApoA-I is safe and effective reducing the levels of cerebral insoluble Aβ and neuroinflammation in an experimental model of AD. Therefore, our results suggest that multifunctional physiological apolipoproteins may be considered as therapeutic molecules in Aβ-associated neuropathologies, proposing the applicability of this family of apolipoproteins (with chaperone function) as therapeutic candidates for protecting the brain in AD. In addition, our data reinforce the notion that peripheral interventions offer non-invasive opportunities to regulate the cerebral β-amyloid load and neuronal loss. Finally, the results generated from this project highlight the involvement of the lipid metabolism in the progression of the disease and encourage further studies to investigate the role of lipoproteins in patients with AD.

4. Publications and Communications

Publications

1) Merino-Zamorano C, Fernández-de Retana S, Montañola A, Batlle A, Saint-Pol J, Mysiorek C, Gosselet F, Montaner J, Hernández-Guillamon M. Modulation of Amyloid-β1-40 Transport by ApoA1 and ApoJ Across an in vitro Model of the Blood-Brain Barrier. J Alzheimers Dis. 2016 May 25;53(2):677-91. doi: 10.3233/JAD-150976.

2) Fernández-de Retana S, Montañola A, Marazuela P, De La Cuesta M, Batlle A, Fatar M, Grudzenski S, Montaner J, Hernández-Guillamon M. Intravenous treatment with human recombinant ApoA-I Milano reduces beta amyloid cerebral deposition in the APP23-transgenic mouse model of Alzheimer's disease. Neurobiol Aging. 2017 Dec;60:116-128. doi: 10.1016/j.neurobiolaging.2017.08.028.

3) Fernández-de-Retana S, Cano-Sarabia M, Marazuela P, Sánchez-Quesada JL, Garcia-Leon A, Montañola A, Montaner J, Maspoch D, Hernández-Guillamon M. Characterization of ApoJ-reconstituted high-density lipoprotein (rHDL) nanodisc for the potential treatment of cerebral β-amyloidosis. Sci Rep. 2017 Nov 7;7(1):14637. doi: 10.1038/s41598-017-15215-w.

4) Fernández de Retana S, Marazuela P, Solé M, Colell G, Bonaterra A, Sánchez-Quesada JL, Montaner J, Maspoch D, Cano-Sarabia M, Hernández-Guillamon M. Peripheral administration of human recombinant ApoJ/Clusterin modulates brain beta-amyloid levels in APP23 mice. Submitted to Alzheimer's Research and Therapy. December 2018.

5) PhD Thesis of Sofia Fernández de Retana, UAB, Barcelona, 11th June 2018.
Title: Study of structural variants of recombinant ApoA-I and ApoJ as a therapeutic approach in the context of cerebral beta-amyloidosis.
Mentor: Mar Hernández Guillamon and Mary Cano-Sarabia
Grade: Excellent cum Laude

Communications

1) Poster presentation at the Cerebral Amyloid Angiopathy International Conference, Boston, US. September 2016.
Title: Synthesis and characterization of rHDL-rApoJ nanoparticles.
Authors: Sofía Fernández-de Retana, Alex Montañola, Jose Luis Sánchez-Quesada, Maialen De-Lacuesta, Joan Montaner, Daniel Maspoch, Mary Cano, Mar Hernández-Guillamon.

2) Poster presentation at the Cerebral Amyloid Angiopathy International Conference, Boston, US. September 2016.

Title: Effect of human recombinant ApoA-I Milano variant on experimental models of cerebral β-amyloidosis
Authors: Alex Montañola, Sofía Fernández-de Retana, Aina Batlle, Maialen De-Lacuesta, Joan Montaner, Mar Hernández-Guillamon.

3) Poster presentation at the X Simposi de Neurobiologia, October 2016, Barcelona.
Title: Synthesis and characterization of rHDL-rApoJ nanoparticles.
Authors: Sofía Fernández-de Retana, Alex Montañola, Jose Luis Sánchez-Quesada, Maialen De-Lacuesta, Joan Montaner, Daniel Maspoch, Mary Cano, Mar Hernández-Guillamon.

4) Short oral presentation (from selected posters) at AD/PD Conference, March 2017, Vienna, Austria.
Title: Recombinant ApoA-I Milano variant reduces cerebral β-amyloid accumulation in a mouse model of Alzheimer's disease
Authors: Mar Hernández-Guillamon, Alex Montañola, Sofía Fernández-de Retana, Aina Batlle, Maialen De-La Cuesta, Joan Montaner.

5) Oral presentation (invited talk) at the Cerebral Amyloid Angiopathy International Conference, September 2018, Lille, France.
Title: Effect of biological variants of ApoJ and ApoA-I in experimental models of cerebral β-amyloidosis
Authors: Mar Hernández-Guillamon, Sofía Fernández-de Retana.

6) Accepted oral presentation at the AD/PD Conference, March 2019, Lisbon, Portugal.
Title: Peripheral administration of human recombinant ApoJ/Clusterin prevents cerebral amyloid angiopathy and the accumulation of insoluble beta-amyloid in APP23 mice
Authors: Mar Hernández-Guillamon, Sofía Fernández-de Retana.