Amyloid formation in Alzheimer's may be modulated by vesicles formed by cholesterol derivatives

Review Article

Anomalous protein aggregation has been pinpointed as a prime cause of a number of serious neurodegenerative diseases that include Alzheimer's, Parkinson's and Creutzfeldt–Jakob. In the case of Alzheimer's, protein aggregation starts with cleavage of an amyloid precursor protein (APP) by a secretase to form amyloid-β (Aβ), a family of peptides that form toxic fibrillar plaques, leading to progressive neuron degeneration. This insight has hastened the search for methods to prevent plaque formation. The cholesterol-rich membrane microdomains that promote secretase activity appear to be involved. Added to that, free cholesterol in the cytoplasm can promote the aggregation of Aβ peptides into fibrils, and cholesterol can interact directly with APP and Aβ amyloid fibrils. With this in mind, Elbassal and colleagues at Florida Atlantic University, USA, investigated how cholesterol and its derivatives could affect the formation of fibrils. They showed that the structural properties of the cholesterol-derivative vesicles, including surface charge and vesicle size, are critical in regulating their effect on Aβ40 aggregation kinetics.

Synthesis of Aβ40 peptide

Amyloid-β comprises peptides of 36–43 amino acids. Aβ40 and the more hydrophobic and thus more potently amyloidogenic Aβ42 have been implicated in Alzheimer's. The central sequence KLVFFAE generates amyloid on its own and probably forms the core of the fibril. The researchers at Florida Atlantic University chose to work with Aβ40 since this peptide has a moderate tendency to aggregate, making it more suitable for studying the effect of cholesterol derivatives on the kinetics of fibril formation compared to the aggressive Aβ42.

The Aβ40 peptide was synthesized on a PS3 Automated Solid Phase Peptide Synthesizer using standard Fmoc strategy. The crude peptide was purified by HPLC using a C_{18} reverse phase column and characterized by matrix-assisted laser desorption ionization (MALDI) mass spectrometry. The Aβ40 peptide used in the kinetic aggregation assay was monomerized by dissolving lyophilized Aβ40 powder in aqueous NaOH followed by sonication in an ice−water bath, and filtration.

Cholesterol interacts with Aβ through hydrophobic interactions

Cholesterol is a neutral and hydrophobic steroid molecule. With a large range of cholesterol derivatives to choose from, the researchers selected amphiphilic cholesterol-SO_4 and cationic DC-cholesterol due to their charged nature.

The team measured aggregation kinetics using a Thioflavin T (ThT) fluorescence assay. ThT selectively binds to the fibrillar structure, which greatly increases its fluorescence quantum yield. The aggregation kinetics of Aβ40 peptide are sigmoidal, with a lag phase followed by an acceleration phase during fibril formation and ending with a plateau (see Figure 1).

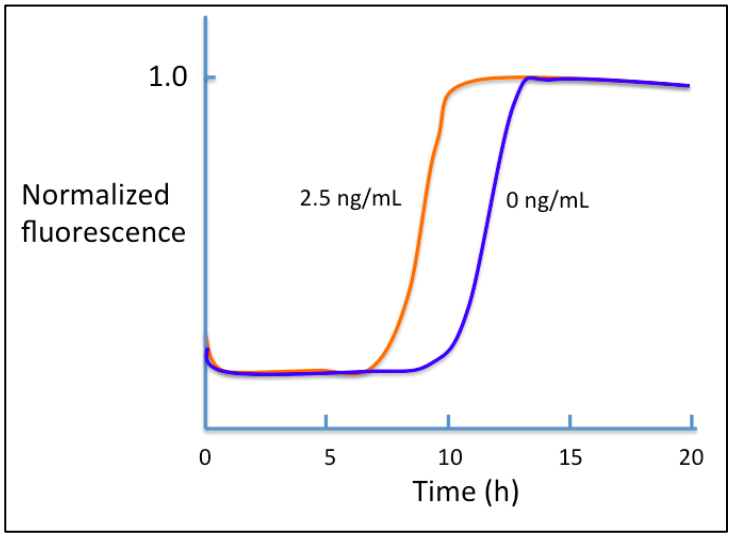

Figure 1. Example of the shift seen in aggregation kinetics of A□40 when adding a low concentration of DC-cholesterol. Based on data shown in Figure 2, Elbassal et al, 2016.

The investigation began with the cholesterol derivatives at concentrations below their critical micelle concentration (CMC), which is the concentration above which micelles are formed. Adding low concentrations of DC-cholesterol, lower than the CMC, speeded up fibril formation, shortening the aggregation half time (t_{50}) by up to 20%. The team saw a similar effect with cholesterol-SO_4. These increases in aggregation rate were equivalent to the effect achieved with cholesterol, which confirmed that adding the cationic group of DC-cholesterol did not interfere with the hydrophobic interactions known to be important in the association between cholesterol and Aβ.

Increasing above CMC leads to new effects

The local concentration of cholesterol-SO_4 in tissues can be much higher than its CMC. The research team therefore looked at the effect of the two cholesterol derivatives on Aβ aggregation when these steroids were presented as aggregated vesicles formed above the CMC. They saw that increasing the concentration of cholesterol-SO_4 dramatically reduced the t_{50} for Aβ aggregation while varying the concentration of DC-cholesterol resulted in a more complicated picture, with considerable reductions in t_{50} at low and medium concentrations followed by a bounce back to high t_{50} at higher concentrations (see Figure 2). In fact, at the highest concentration of 250 µg/mL, DC-cholesterol strongly *inhibited* Aβ fibrillization.

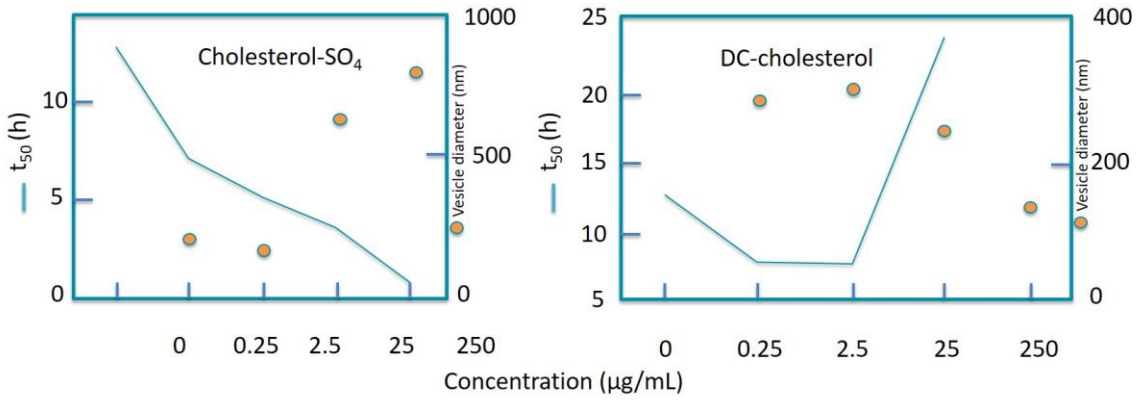

Figure 2. Changes in concentration, which also affects vesicle size, play a major role in the effect of the cholesterol derivatives on Aß fibrillization. Based on data from Figures 4 and 6, Elbassal et al, 2016.

Vesicle size matters

Measuring vesicle size clearly showed that the effect of the cholesterol derivatives on Aβ fibrillization proves to be a complex interplay between vesicle size and surface charge. For example, the dramatic effect of high concentrations of anionic cholesterol-SO_4 in boosting the aggregation of Aβ40 can be related to its ability to form lipid vesicles that, *"provide an interface that can act as a catalytic or inhibiting surface and can influence the Aß aggregation kinetics in a similar way as nanoparticles"*. This could be due to the ability of the sulfate group to steer binding between Aβ and cholesterol-SO_4 vesicles through electrostatic interactions.

A step forward in the inhibition of fibril formation

Summing up, the researchers suggest that, *"the characteristics of the protein–vesicle interface, including the surface charge properties and the size of the cholesterol derivative vesicles, are critical in regulating the effects of the vesicles of these cholesterol derivatives on Aß40 amyloid formation*". Such insights could well lead to new breakthroughs in the treatment of some of the most distressing diseases we are fighting in our aging population.

Reference

Effects of Charged Cholesterol Derivatives on Aβ40 Amyloid Formation. Elbassal EA, Liu H, Morris C, Wojcikiewicz EP, Du D. J Phys Chem B. 2016 Jan 14;120(1):59-68. doi: 10.1021/acs.jpcb.5b09557. Epub 2015 Dec 23.