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CHOLESTEROL AND Aβ-INDUCED MITOCHONDRIAL OXIDATIVE STRESS AS A CAUSAL FACTOR AND THERAPEUTIC TARGETS IN ALZHEIMER'S DISEASE

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1. Summary

Increased cholesterol has been consistently linked to amyloidogenesis. Recent evidence suggested that cholesterol might have an even bigger impact on Alzheimer's disease (AD), because cholesterol could also regulate armload beta (Aβ) clearance. Our previous results showed that cholesterol accumulation in mitochondria accelerates AD-like symptoms, via decreasing mitochondrial glutathione (GSH) levels and promoting Aβ-induced oxidative stress. In the same line, oxidative inactivation of Aβ-degrading enzymes and impairment of microglia phagocytosis by reactive oxygen species (ROS) had been described. Moreover, our preliminary studies suggested that changes in the transcriptional activity of the amyloid protein precursor intracellular domain (AICD) could play a role in disturbances of cholesterol homeostasis and Aβ metabolism. Based on these data, we hypothesized that cholesterol contributes in part to Aβ load by adversely affecting AICD function and other key components of cellular degradation systems. To test this hypothesis we used brain homogenates and neurons from middle-age APP/PSEN1 mice that overexpress the sterol-related transcription factor SREBP-2, and cultured cells treated with cholesterol/GSH-modifying agents.

Our results provide a deeper understanding of the interrelationship between cholesterol and Aβ accumulation, uncovering the dual ability of cellular cholesterol to enhance Aβ synthesis and aggregation while impairing its elimination. We have demonstrated that cholesterol can affect different cellular mechanisms of Aβ clearance, including autophagy, amyloid proteolytic cleavage and microglial phagocytosis. We have shown that mitochondrial oxidative stress potentiated by cholesterol is a contributory factor of defective Aβ elimination, through affecting the function of Aβ-degrading enzymes. The enhanced Aβ clearance that we have observed after the in vivo recovery of mitochondrial GSH by GSH ethyl ester further highlights the key role of mitochondrial oxidative stress in Aβ degradation, providing a new therapeutic strategy for AD treatment. In parallel, we have identified short fatty acids (FA) present in coconut oil as dietary molecules which can strongly increase the activity of IDE (insulin degrading enzyme), one of the main Aβ-degrading enzymes. The fact that FA and GSH ethyl ester administration can be readily adjustable for human consumption supports that this experimental interventions could be of in vivo relevance.

2. Results

- Cholesterol regulates the expression and activity of Ab-degrading enzymes such as IDE (insulin degrading enzyme) and NEP (neprilysin). An increase in intracellular cholesterol, by depleting mitochondrial GSH levels, results in oxidative inhibition of both enzymes. At the same time, the increase in cellular cholesterol favours the release of IDE by extracellular vesicles. Secretion of IDE is autophagy-dependent and is regulated by cholesterol-induced changes in the autophagy flow. Recovery of the mitochondrial GSH by treatment with the soluble GSH form (GSH ethyl ester) restores the enzymatic activity of both enzymes and improves the proteolytic degradation of extracellular Aβ. In contrast, we have observed that unlike intracellular cholesterol, a rise of cholesterol levels in the medium has an anti-amyloidogenic role, favouring the function of extracellular IDE.

- In parallel, we have screened fatty acids (FA) to identify molecules that can counteract the decreased Aβ degradation in the pathological AD situation. We have identified FA shorter than C16 with the potential to increase Aβ degradation dependent of IDE. APP/PSEN1 mice fed with coconut oil (highly enriched with short-chain FA such as lauric acid (12:0) and myristic acid (14:0)) showed increased IDE activity compared to control-fed animals, with reduced brain Aβ levels.

- Cholesterol impairs the elimination of intracellular TAU and Aβ through autophagy, while promoting Aβ secretion to the extracellular environment. High cholesterol levels stimulate autophagosome formation as a result of an enhanced mitochondrial oxidative stress, but block their correct fusion with lysosomes.

- Cholesterol regulates mitophagy (elimination of defective mitochondria through autophagy), as well as mitochondrial biogenesis. Increased cholesterol levels promote the recruitment of mitophagy key proteins such as PINK1 and PRKN, and decreases the expression of PGC-1α.

- Cholesterol (through promoting Ab-induced mitochondrial oxidative stress) can also regulate inflammasome induction, and the consequent inflammatory response, thereby affecting neuronal viability and microglial phenotype (pro-inflammatory vs. phagocytic).

- Aβ metabolism regulates cholesterol levels: The AICD product resulting from the amyloidogenic processing of the amyloid precursor protein (APP) lowers the expression of key enzymes in the synthesis of cholesterol, such as 3-hydroxy-3-methyl-glutaryl-CoA synthase 1 (HMGCS1), 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR), and farnesyl diphosphate synthase (FDPS). These studies have allowed us to identify a cascade of kinases that link Ab with the mitochondrial function and activity of the HMGCR enzyme.

- Cholesterol induces Aβ synthesis by directing amyloidogenic proteins to membrane areas called "lipid rafts" and favouring the processing of APP.

3. Relevance and future practical applications

Our systematic analysis of the impact of cholesterol in different cellular processes shows that cholesterol-enhanced oxidative stress can alter Aβ synthesis and impair different processes responsible for Aβ removal, including Aβ-degrading enzymes, autophagy and microglia phenotype. With these studies, we have gained a better understanding of these processes; we have identified mitochondrial oxidative stress and the maintenance of mitochondrial GSH levels, the main antioxidant defence of mitochondria, as key regulatory factors in the elimination of Aβ. We also believe that we have established the basis to improve clearance-related therapeutic strategies focused to reduce Aβ levels, particularly, the soluble and most toxic Aβ species. Our experimental results in cell lines and AD mouse models suggest that a dietary supplementation with GSH ethyl ester (a soluble compound capable of recovery the mitochondrial GSH content) or with short-chain FA (like those present in coconut oil), by recovering the endogenous mechanisms of Aβ clearance, could have beneficial effects in the treatment of Alzheimer's disease.

4. Literature

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