

Title	アルツハイマーアミロイド の凝集と膜相互作用におけるコレステロールと酸化コレステロールの影響
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## Abstract

Cholesterol is the most abundant animal steroid, consisting of four fused, rigid rings, a polar hydroxyl group at C3, and a branched, nonpolar iso-octyl side chain at C17 of ring structure. The most important function of cholesterol is to be a vital structural constituent and physicochemical property modulator of cell membranes. In addition, the sterol serves as the metabolic precursor of steroid hormones and bile acids, and it is essential for neuronal activities and functions. Cholesterol is a susceptible target of cellular oxidation induced by enzymes and reactive oxygen species (ROS), generating two main categories of oxidized derivatives (oxysterols) which are (i) those oxygenated on the side-chain and (ii) those oxygenated on the sterol ring. Compared to cholesterol, oxysterols have an additional oxygen group that renders oxysterols relatively more hydrophilic and different from cholesterol in the orientation in membranes. Oxysterols exhibit both positive and negative biological roles. Some of them at physiological concentrations play an important impact in cholesterol homeostasis, sterol biosynthesis, and cell signalling, while others have harmful effects and contribute to some human diseases. Both cholesterol and oxysterols have been widely implicated in Alzheimer's disease, the most common neurodegenerative disorders in humans. Accumulating evidences have demonstrated that the accumulation, aggregation and cytotoxicity of amyloid beta (A $\beta$ ) peptide in the brain are key processes in the pathogenesis of the disease. The interaction of A $\beta$  with cell membranes plays crucial roles in these processes. Cholesterol, with the function as an essential component and property modulator of cell membranes, remarkably alters A $\beta$ /membrane interaction. However, the role of cholesterol as a protective factor or a deleterious agent in A $\beta$ /membrane interaction remains controversial. In addition, the impact of oxysterols in this interaction is not fully understood although these compounds are reported to have high abilities to change cell membrane properties.

This dissertation aimed to investigate the effect of cholesterol and oxysterols on the interaction of A $\beta$  with the lipid bilayer of membranes and membrane-mediated aggregation of the peptide. Three membrane systems different in the level of complexity, including homogeneous, heterogeneous model membranes and actual cell membranes, were used. First, I clarified how cholesterol and two commonly occurring and reportedly harmful oxysterols, 7-ketocholesterol (7keto) and 25-hydroxycholesterol (25OH), influence the interaction of A $\beta$ -40 and A $\beta$ -42 aggregated species with the lipid bilayer of a homogenous membrane system and associated dynamics of the membrane. Second, the localization of A $\beta$ -42 protofibrils, which is widely reported to be a harmful species, in lipid lateral compartments of a heterogeneous model membrane in presence of cholesterol and 7keto was unravelled. Third, I further advanced my study on the link between these compounds and A $\beta$ /membrane interaction by using living cells. Last, the influence of cholesterol and 7keto on kinetics of A $\beta$  aggregation mediated by model membranes, and the morphology and biological membrane interaction of some A $\beta$  aggregates were investigated.

The studies on homogeneous model membranes clearly show that oxysterols mediated localization of A $\beta$  in membranes and the peptides-induced membrane dynamics, in contrast to role of cholesterol in inhibiting A $\beta$ /membrane interaction. The effect of 7keto and 25OH are different due to distinct positions of the additional oxygen group in their structures. The former induced a high propensity of membrane toward association with A $\beta$ , while the latter made membrane more capable of morphological changes in response to the peptide. Comparing two common A $\beta$  isoforms, A $\beta$ -42 protofibrils were more interactive with homogeneous membranes than A $\beta$ -40 species. These findings suggest the inhibitory effect of cholesterol and enhancing influence of oxysterols on the interaction of A $\beta$  with the lipid bilayer of membranes.

In heterogeneous model membranes, which retain the lateral lipid organization of cell membranes, cholesterol decreased the localization of A $\beta$ -42 protofibrils in solid-ordered domains and increased that in liquid-ordered domains. The sterol changed the amount of A $\beta$  associating with liquid-disordered (Ld) phase in different tendencies depending on the composition of heterogeneous membrane systems. These effects were attributed to cholesterol's capability of altering the fluidity of lipid phases. On the other hand, 7keto mainly enhanced the fluidity and interaction of protofibrillar A $\beta$ -42 with Ld phase. These results demonstrate the impact of cholesterol in directly modulating A $\beta$

interaction with lipid domains of membranes in addition to its effect on A $\beta$ /GM1 binding as reported previously. They also indicate the harmful impact of cholesterol oxidized derivatives which promotes A $\beta$  association with heterogeneous membranes.

Jurkat T cell, a kind of white blood cell and a target of A $\beta$ , was used to assess the effect of cholesterol and 7keto on protofibrillar A $\beta$ -42/cell-membrane interaction. I found that the loss of membrane cholesterol strongly enhanced the interaction of A $\beta$ -42 protofibrils with Jurkat T cells and decreased the viability the cells exposed to the protofibrils compared to cells with basal cholesterol content. Conversely, the increase in cholesterol content did not significantly change these processes. On the other hand, 7keto had a high ability to enhance the localization of A $\beta$ -42 protofibrils in Jurkat T cell membranes and increase the effects of the peptide which reduce cell viability and increase cytosolic Ca<sup>2+</sup> content of the cells. These influences of cholesterol and 7keto were discussed based on their ability to change membrane fluidity as indicated by studies on model membranes. The results suggest that cholesterol has the beneficial role in A $\beta$ -induced toxicity to Jurkat T cells, in agreement with previous studies on neuronal cells, while 7keto may be a harmful factor in this process.

Regarding to A $\beta$ -42 aggregation mediated by model membranes, the strikingly different effects of cholesterol and 7keto were demonstrated. The presence of cholesterol in DOPC vesicles moderately inhibited the kinetics of nuclei formation and considerably accelerated fibrillar A $\beta$ -42 growth. However, the formation of nuclei from monomers was slightly increased and fibril elongation was remarkably inhibited by the partial substitution of membrane cholesterol with 7keto. Moreover, cholesterol-containing vesicles induced a faster formation of fibrils which has a low propensity to cells, while 7keto-containing vesicles inhibited the formation of fibrils, maintain the peptide in protofibrillar aggregates which were highly able to localize in cells. Since the cytotoxicity of A $\beta$  remarkably depends on the aggregated state, these results suggested that cholesterol hinders A $\beta$  cytotoxicity to cells by accelerating the formation of fibrils, while 7keto mediates A $\beta$  cytotoxicity by inhibiting the conversion of protofibrils to mature fibrils.

In conclusion, I have shown that cholesterol has a protective role and oxysterols, in particular 7keto, are risk factors in A $\beta$ -induced cytotoxicity. The effect of cholesterol and oxysterols is associated with to their abilities to alter interaction of A $\beta$  with membranes and fibrillation of the peptide mediated by membranes. In general, cholesterol inhibited A $\beta$ /membrane interaction and accelerated the formation of A $\beta$  fibrils which are less harmful to cells than other aggregate species. Conversely, oxysterols enhanced the interaction and hindered A $\beta$  fibrillation, thereby maintaining the existence of A $\beta$  protofibrils, widely reported to be a harmful species. As far as I am aware, this dissertation is the first systematic study about the effect of cholesterol oxidative derivatives on A $\beta$ /membrane interaction. The findings of this dissertation are important to clarify the impact of oxidative stress in A $\beta$ -induced cytotoxicity and neuroinflammation in the pathogenesis of Alzheimer's disease. They also suggest that prevention and/or repair of oxidative stress by antioxidants and reduction of ROS generation may be a potential approach in the treatment of Alzheimer's disease.

**Keywords:** Cholesterol, Oxysterols, Amyloid-beta/membrane interaction, Amyloid-beta aggregation, Alzheimer's disease