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The Role of Cholesterol in Pathogenesis of Alzheimer's Disease

Dual Metabolic Interaction between Amyloid β-Protein and Cholesterol

Makoto Michikawa*

Department of Dementia Research, National Institute for Longevity Sciences, 36-3 Gengo, Morioka, Obu, Aichi 474-8522, Japan

Abstract

The implication that cholesterol plays an essential role in the pathogenesis of Alzheimer's disease (AD) is based on the 1993 finding that the presence of apolipoprotein E (apoE) allele ε4 is a strong risk factor for developing AD. Since apoE is a regulator of lipid metabolism, it is reasonable to assume that lipids such as cholesterol are involved in the pathogenesis of AD. Recent epidemiological and biochemical studies have strengthened this assumption by demonstrating the association between cholesterol and AD, and by proving that the cellular cholesterol level regulates synthesis of amyloid β-protein (Aβ). Yet several studies have demonstrated that oligomeric Aβ affects the cellular cholesterol level, which in turn has a variety of effects on AD-related pathologies, including modulation of tau phosphorylation, synapse formation and maintenance of its function, and the neurodegenerative process. All these findings suggest that the involvement of cholesterol in the pathogenesis of AD is dualistic—it is involved in Aβ generation and in the amyloid cascade, leading to disruption of synaptic plasticity, promotion of tau phosphorylation, and eventual neurodegeneration. This review article describes recent findings that may lead to the development of a strategy for AD prevention by decreasing the cellular cholesterol level, and also focuses on the impact of Aβ on cholesterol metabolism in AD and mild cognitive impairment (MCI), which may result in promotion of the amyloid cascade at later stages of the AD process.

Index Entries: Alzheimer's disease; cholesterol; amyloid β-protein; tau phosphorylation; statin; HMG-CoA reductase inhibitor; raft.

* Author to whom all correspondence and reprint requests should be addressed. E-mail: michi@nils.go.jp

Introduction

The brain is the most cholesterol-rich organ in the human body. However, cholesterol metabolism in the central nervous system (CNS) is not fully understood. Because the CNS is segregated from the systemic circulation by the blood-brain barrier, lipids transported by the systemic circulation are not generally available to the CNS. Moreover, the CNS contains high-density-lipoprotein (HDL)-like particles, but does not contain low-density lipoprotein (LDL) or very low-density lipoprotein (VLDL) *(1)*, and it contains fewer types of apolipoproteins than the systemic circulation. Apolipoproteins identified in cerebrospinal fluid (CSF) are mainly apolipoprotein E (apoE) and apoA-I associated with small HDL *(2–4)*. These lines of evidence indicate that there is a distinct system for cholesterol metabolism in the CNS that is independent of that in the systemic circulation. ApoE is one of the major apolipoproteins that regulates cholesterol metabolism in the CNS by promoting the release of cellular cholesterol to generate HDL-like particles and by the uptake of these HDL particles via apoE receptors *(1,3–7)*.

The discovery that the presence of apoE allele ε4 is a strong risk factor for the development of Alzheimer's disease (AD) *(8–11)* suggests the involvement of apoE and its metabolite, cholesterol, in AD pathogenesis. AD is a slowly progressive neurodegenerative disease, pathologically characterized by the extracellular accumulation of senile plaques—the major component of which is amyloid β-protein (Aβ)—and the intracellular formation of neurofibrillary tangles (NFTs), which are composed of hyperphosphorylated tau *(12)*. Biochemical and morphological analyses of AD suggest the involvement of early synaptic dysfunction followed by its subsequent progression, including increased synaptic loss, neurite dystrophy, formation of NFTs, and eventual neuronal death *(13,14)*. The mechanism underlying this progression is widely believed to be initiated and promoted by an aggregated Aβ, which is known as the amyloid cascade hypothesis *(15,16)*. Thus, it is reasonable to determine the roles of apoE and lipids—including cholesterol, whose metabolism is regulated by apoE—in the pathogenesis of AD from the viewpoints of their effect on Aβ generation and AD-related pathologies such as synaptic damage, tau phosphorylation, and neuronal death. A growing body of evidence suggests the association and underlying mechanism(s) of these two factors. However, some conflicting issues must be resolved regarding the putative association between the two. In this article, recent studies that describe the association between cholesterol and AD pathophysiology are categorized into several groups according to the research aspect and assumption; a high or low cholesterol level promotes or prevents AD pathogenesis, respectively.

Association of Elevated Cholesterol Level in Serum or Brains with a Risk of Developing AD

Several epidemiological studies have shown that an increased serum cholesterol level during the long-preclinical phase is correlated with the development of AD *(17–19)* and mild cognitive impairment (MCI) *(20)*. Based on these findings, it is assumed that a reduction in the serum cholesterol level could reduce the prevalence of AD. In support of this theory, recent studies have shown that the prevalence of AD in patients taking statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, is significantly reduced compared with that in a total patient population or patients taking other medications *(21)*, and that the current use of statins reduced the risk of dementia *(22)*. These findings suggest that a high level of serum cholesterol could elevate the CSF cholesterol level, which may lead to the development of AD. However, a previous study indicates that there is no correlation between the level of serum total cholesterol and that of CSF total cholesterol *(23)*, suggesting that the association is complex, and that

further studies are needed to determine this. In the case of statins, their inhibitory effect on AD may not be attributable only to the decrease in the CSF cholesterol level following the decrease in the serum cholesterol level, but also to the decrease in the cellular cholesterol level. In support of this theory, a recent study showed that statins directly affect cholesterol metabolism in the human brain *(24)*. It was demonstrated that the levels of 24S-hydroxycholesterol—for which conversion occurs mainly in the brain—in the plasma are reduced in patients using high-dosage simvastatin *(24)*. Eckert et al. presented more direct evidence that the brain cholesterol level significantly decreased following lovastatin treatment in mice *(25)*. These findings suggest that statins could be directly involved in the prevention of AD development by decreasing the cellular cholesterol level in the brain. However, statins have various biological effects in addition to the inhibition of cholesterol synthesis. These include protection against nitric oxide (NO) as well as oxidative stress, and anti-inflammatory and anti-platelet effects *(26)*, prompting treatment alternatives to reducing cholesterol levels, which could explain the reduction in the prevalence of AD among those taking statins. This idea may raise criticism based on the interpretation of findings that focus on a causal relationship between statins and AD or cholesterol and AD by highlighting the inconsistency in effects of different statins with similar brain-blood-barrier permeabilities and similar inhibitory effects on cholesterol synthesis *(21)*. However, as the authors have suggested, the duration of treatment with statins rather than any other factors may have caused this inconsistency, because other studies have shown a difference in risk of AD based on a long preclinical history of elevated serum cholesterol levels, but not on current cholesterol levels *(17,18)*.

Several biochemical studies have explored the molecular mechanism(s) by which AD development may be prevented, revealing that cellular cholesterol levels regulate the metabolism of amyloid precursor protein (APP) as well as Aβ synthesis and its secretion both in vitro and in vivo. Previously, cholesterol was shown to modulate processing of APP in cultured neurons *(27–29)*. The groups of Bodovitz and Klein *(27)*, and Racchi et al. *(28)* showed that cholesterol modulates α-secretase cleavage of APP, and the level of cellular cholesterol is inversely correlated with the amount of soluble APP, an N-terminal fragment of APP cleaved by α-cleavage *(27)*. Another recent study showed that a decreased cellular cholesterol level promotes the nonamyloidogenic pathway (α-secretase activity) *(30)*. Other studies demonstrated that when the cellular cholesterol level in neurons decreases following treatment with an 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor with or without additional treatment with methyl-β-cyclodextrin, the amount of Aβ released into the culture media markedly decreases *(29–31)*. It was shown that a decreased cellular cholesterol level inhibits the amyloidogenic pathway (β-and g-secretase activity) *(29)* and promotes the nonamyloidogenic pathway (α-secretase activity) *(27,28,30)*. In particular, Fassbender's study suggests that the mechanism by which statins reduce the risk of AD is associated with the reduced production of Aβ in the brain *(31)*. The detailed mechanism underlying the putative association between cholesterol and Aβ generation has not yet been elucidated, but one possible explanation for this association is that the cholesterol-rich domain, known as the lipid raft, is one of the key domains generating Aβ. Since it was shown that APP in neurons is associated with lipid rafts *(32,33)*, that cholesterol depletion decreases APP association with these rafts *(29)*, and that the sites of γ-secretase activity and Aβ generation are associated with cholesterol-rich microdomains *(34)*, it may be possible that Aβ generation requires raft integrity and a lipid component as optimal conditions. Therefore, an alteration in raft components could change the configuration of either the enzymes or the substrate associated with the rafts, leading to an alteration in Aβ generation. Since cholesterol is an essential component for

generating lipid rafts, cholesterol depletion in cells induces disruption of the structure and function of lipid rafts in which amyloidogenic processing of APP occurs to generate Aβ, and also alters the processing of APP. However, one may note a discrepancy between studies using cultured cells and those using animals. The significant reduction in the generation and secretion of Aβ was demonstrated in cultures, in which at least 50% cholesterol was depleted *(29,30)*, and although the amount of secreted Aβ in the CSF was reduced, the total brain cholesterol levels were not significantly reduced in statin-treated guinea pigs *(31)*. These findings suggest another possibility—that a reduction of Aβ secretion or reduction in the prevalence of AD resulting from statin treatment may not be associated with cholesterol, but may involve nonsterol mechanisms. Further studies are needed to clarify these issues.

Association of Decreased Level of Serum, CSF, or Cellular Cholesterol with a Risk of Developing AD

Several studies have revealed conflicting findings: cholesterol levels in serum, cell membranes of the brain, and CSF decreased in AD patients compared to those in controls *(35–39)*, and increased dietary cholesterol levels reduced Aβ secretion *(40)*. However, there are no definitive data across studies to indicate that the level of total brain cholesterol differs between AD patients and normal individuals. A few studies showed that cholesterol in the plasma membrane acts as a modulator of the Aβ effect on brain membranes *(41)* and Aβ neurotoxicity by modulating the membrane insertion of Aβ. At a low cholesterol level, Aβ remains on the membrane surface mainly in a β-sheet structure, leading to exhibition Aβ neurotoxicity *(42)*; and a high cholesterol level in the plasma membrane results in a decreased level of Aβ-cell-surface binding and subsequent cell death *(42,43)*. The increased cholesterol level in the membrane was reported to attenuate the disordering effect of Aβ on brain membranes *(44–46)*. It has also been suggested that apoE4, one of the strongest risk factors for AD, may contribute to disturbances in lipid metabolism that finally lead to a low cholesterol level in the AD brain *(47)*. These lines of evidence, together with the results described in the Introduction to this article, indicate that the relationship between the alteration in cholesterol metabolism and AD pathogenesis still remains controversial. These conflicting results may direct attention to our target: which cholesterol level is altered, that of the physiological fluid (serum or CSF), an organ (brain), cells (neurons or glial cells), organelles (including the plasma membrane, endoplasmic reticulum [ER], Golgi), or microdomains (lipid rafts or others). Depending on the subject, the role of cholesterol, and thus the effect of the alteration of its level, should be different. For example, cholesterol accumulation is observed in Niemann-Pick disease, type C, and the total cholesterol level in cells—particularly in late endosomes and lysosomes—is elevated. However, the total cholesterol level in specific cell compartments such as caveolae *(48)* and detergent-insoluble, low-density membrane fraction (our unpublished data) is reduced. Another example is a study showing cholesterol accumulation in mature senile plaques of AD brains and in transgenic APPsw mouse brains *(49)*. This study supports the theory that an elevated level of cholesterol is associated with AD pathogenesis. However, another possible interpretation is that cholesterol accumulation in senile plaques may induce cholesterol deficiency in specific domains as a result of repartitioning of cholesterol from areas in which it plays a normal physiologic role in brain regions. This theory is supported by recent findings demonstrating that oligomeric Aβ promotes cholesterol release resulting in the generation of HDL-like particles that cannot be internalized by neurons *(50)*, eventually leading to a reduction in the cholesterol level

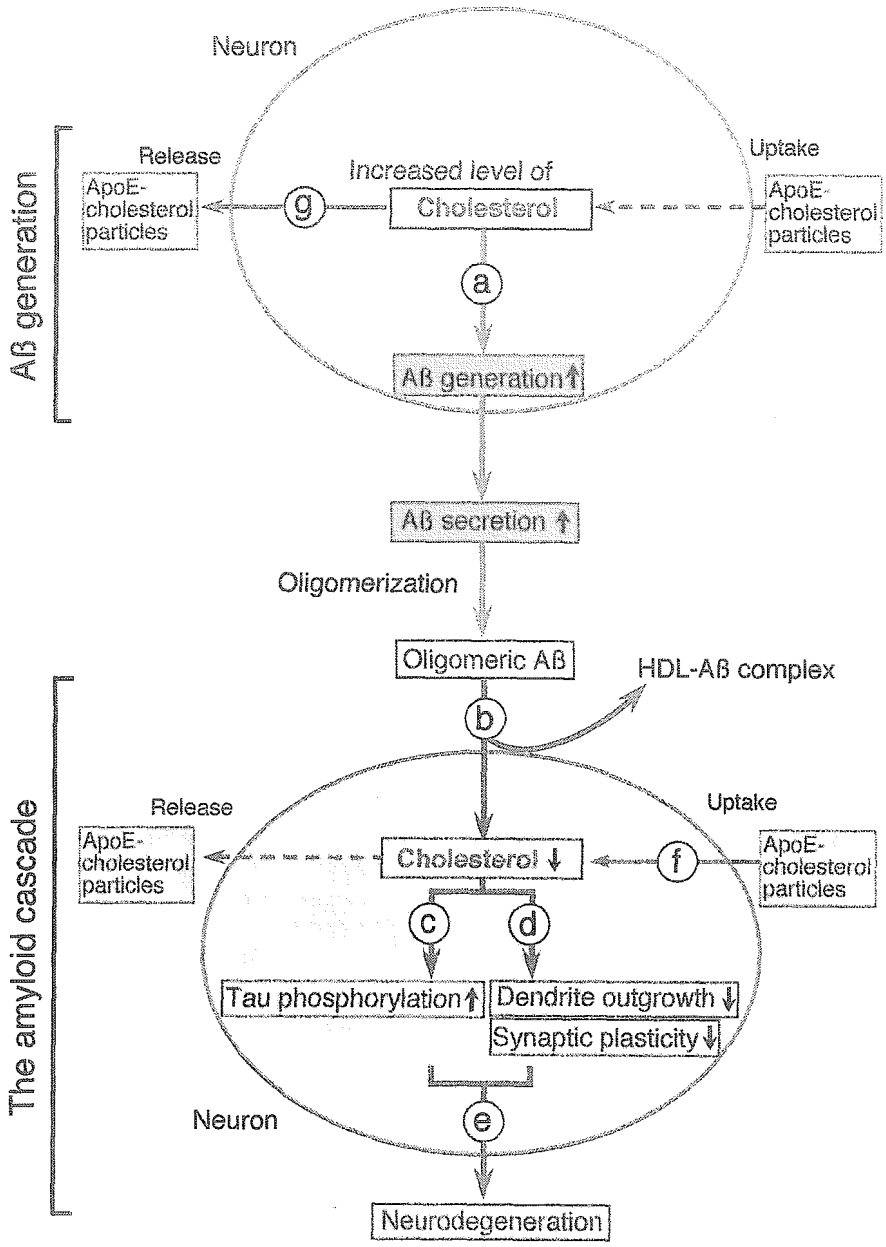
in neurons *(51)*. These studies suggest that cholesterol associated with oligomeric Aβ may be accumulated extracellularly, while the intracellular cholesterol level decreases. Thus, further studies are needed to elucidate the association between cholesterol and the mechanisms promoting AD pathology.

The Critical Role of Cholesterol in the Amyloid Cascade

The previously described studies and those that follow in this section have defined the role of cholesterol in the amyloid cascade in a hypothesis explaining the mechanism underlying the amyloid cascade theory—Aβ accumulation leads to AD-related pathologies including tau phosphorylation, synaptic loss, and neurodegeneration. Fig. 1 summarizes the findings that focus on the involvement of cholesterol in the pathogenesis of AD and Aβ generation. As mentioned in the second section of this article, the cellular level of cholesterol modulates Aβ synthesis and secretion (Fig. 1a). When the concentration of monomeric Aβ increases, Aβ is assumed to form oligomers under physiological conditions, particularly in the case of Aβ1-42 *(52)*. Oligomeric Aβ was shown to promote lipid release from neurons, resulting in the generation of HDL-like particles and inhibition of cholesterol synthesis, which eventually led to a reduction in the cellular cholesterol level (Fig. 1b).

The Role of Cholesterol in Synapse Formation

This Aβ-mediated alteration in cellular cholesterol homeostasis presumably leads to synaptic dysfunction, because cholesterol from glial cells as apoE-containing lipoproteins has been shown to play a critical role in the formation of mature synapses *(53)*. Cholesterol as apoE-lipid complex generated by astrocytes was shown to be the limiting factor that regulates synapse formation and its functions. The dependence of synapse formation on astrocyte-derived cholesterol is also supported by a previous study showing that most synapses in the developing brain are formed coincidentally with the development of astrocytes *(54)*. The importance of cholesterol in maintaining synapse formation has been investigated by modulating cellular cholesterol levels. Cholesterol was suggested to contribute to modulation of cellular kinases and phosphatases in neurons, and a decrease in the level of cholesterol results in a dendrite-specific inhibition of neurite outgrowth *(55,56)*. In the CNS, apoE is one of the major apolipoproteins regulating cholesterol metabolism and is mainly synthesized and secreted as apoE-lipid particles from astrocytes; the ability of astrocytes as a cholesterol supplier may be partially dependent on apoE *(2,4)*. Thus, it is reasonable and also important to study the isoform-dependent contribution of apoE to AD development from the perspective of the isoform-dependent function of apoE in the regulation of cholesterol. Cellular cholesterol metabolism is regulated by endogenous cholesterol biosynthesis, uptake of apoE-containing lipoproteins via apoE receptors, and cholesterol release by lipid acceptors such as apoE. Recently, we discovered an apoE-isoform dependence for one of these functions; that is the ability of apoE3 to promote cholesterol release is greater than that of apoE4 *(6)* (Fig. 1g). Although the role of apoE in AD pathogenesis remains unclear, this isoform-specific cholesterol release from neurons may result in a higher cholesterol level in CNS neurons of apoE4 carriers than in those of apoE3 carriers, leading to increased generation of Aβ in the CNS of apoE4 carriers (Fig. 1g). However, we also found that apoE3-expressing astrocytes generate more HDL-like particles than apoE4-expressing astrocytes with similar amount of apoE molecule *(7)*, implying that apoE3-expressing astrocytes can supply more cholesterol to neurons (Fig. 1f) for use in regeneration and for synaptic plasticity in neurons. Further studies are required to address these issues.



The Role of Cholesterol in Tau Phosphorylation

The effect of Aβ on cellular cholesterol metabolism has also been investigated. Liu et al. *(57)* showed that Aβ alters intracellular vesicle trafficking and cholesterol homeostasis. Our recent studies showed that oligomeric—but not monomeric—Aβ affects cholesterol metabolism, and that oligomeric Aβ promotes cholesterol release, resulting in generation of HDL-Aβ particles that cannot be

Fig. 1. Putative roles of cholesterol in AD pathogenesis. The involvement of cholesterol in AD pathogenesis may be dualistic; it is involved in Aβ generation (blue lines) and in the amyloid cascade (red lines). ApoE-cholesterol complexes serve to maintain the homeostasis of cellular cholesterol metabolism by the uptake and release of cholesterol in an isoform-specific manner (green lines). For the effect of cellular cholesterol level on Aβ generation, the increased levels of cellular cholesterol promote the generation and subsequent secretion of Aβ, and the decreased levels of cellular cholesterol following statin treatment attenuate them **(a)**. For the role of cholesterol in the amyloid cascade, an increasing amount of extracellular Aβ leads to the formation of oligomers by a still undetermined mechanism, which in turn reduces the cellular cholesterol level by promoting cholesterol release and inhibiting cholesterol synthesis **(b)**. Cholesterol deficiency was shown to induce tau phosphorylation and inhibit synapse formation, which may lead to neurodegeneration (**c, d,** and **e**). ApoE contributes to the maintenance of cholesterol homeostasis in neurons by two mechanisms: cholesterol release from neurons **(g)** and its uptake into neurons **(f)**. The mechanism by which the apoE isoform specifically contributes to AD development remains undetermined. However, the fact that extracellular apoE3 has a stronger ability as a cholesterol acceptor than apoE4 *(6)* **(g)** suggests that apoE may be involved in the isoform-dependent, increased level of cholesterol, which may affect Aβ generation. We also found that endogenous apoE3 synthesized in astrocytes can generate more HDL-like particles with less apoE than apoE4 *(7)* **(f)**, implying that apoE3-expressing astrocytes can supply more cholesterol to neurons than apoE4-expressing astrocytes, and thereby supporting neuronal plasticity and promoting neurodegeneration. Under these conditions, cholesterol demand of neurons markedly increased.

internalized by neurons *(50)*, and subsequently reducing cellular cholesterol levels *(51)*. In addition, recent studies demonstrated the relationship between tau phosphorylation and cholesterol. When the cellular cholesterol level decreases following treatment with HMG-CoA reductase inhibitors or β-cyclodextrin, tau phosphorylation is enhanced in cultured neurons *(58)* and in hippocampal-slice cultures *(56)*. The link between alteration in cholesterol metabolism and the promotion of tau phosphorylation was also supported by the finding that tau is hyperphosphorylated in the brains of the NPC and NPC model mouse, in which cholesterol metabolism is altered because of the lack of the NPC1 protein *(59)*. The promotion of tau phosphorylation in NPC was suggested to be the result of a cholesterol deficiency in a specific compartment in the plasma membrane *(59,60)*, despite an elevated total cellular cholesterol level. In support of these findings, it was shown that in NPC-deficient cells, the cholesterol levels in the detergent-insoluble, low-density membrane fractions—also called lipid rafts, caveolae, or detergent-insoluble glycosphingolipid-rich domains (DIGs)—decrease *(48)*. These findings suggest that the extracellular accumulation of Aβ and subsequent formation of oligomeric Aβ affect cholesterol metabolism in neurons, leading to reduced cholesterol levels in the plasma membrane, particularly in lipid rafts, a critical domain for signal pathways *(61,62)*, which in turn affects raft function and leads to tau phosphorylation.

Considering all the findings described in the first three sections of the article, it is possible that the involvement of cholesterol in AD pathogenesis is dualistic: The elevated levels of cellular cholesterol contribute to AD development by elevating Aβ secretion; however, the increased amount of oligomerized Aβ reduces cellular cholesterol levels, which in turn may promote AD progression.

Another Role of Cholesterol in Aβ Aggregation

Several groups have proposed another putative role for cholesterol in AD pathogenesis, claiming that cholesterol is one of the key molecules in the fibril formation of Aβ. Because the oligomeric and aggregated Aβ are assumed to play a critical role in the amyloid cascade, the conversion of soluble, nontoxic

Aβ to oligomeric and aggregated Aβ is the critical step in AD development. A recent paper showed that increased cholesterol levels in the lipid bilayers facilitate the binding of Aβ to the membranes, and an increase in the membrane-bound Aβ concentration triggers the promotion of conformational change from a helix-rich to a β-sheet-rich structure, becoming an endogenous seed for amyloid formation *(63)*. The cholesterol-dependent generation of Aβ seeds was demonstrated, and an increased level of cholesterol synthesis increase was shown to the amount of Aβ seeds in a conditioned medium for Madin-Darby canine kidney (MDCK) cells *(64)*. A previous study also suggested the critical role of cholesterol in Aβ fibril formation by demonstrating that the generation of GM1 ganglioside-bound Aβ (GM1/Aβ) is enhanced by the combination of cholesterol and sphingomyelin in membranes in proportions similar to those in the lipid rafts *(65)*. Since GM1/Aβ was reported to accelerate amyloid fibril formation *(66,67)*, these findings suggest that the cellular cholesterol level—particularly in the cholesterol-rich domain such as the rafts—affects the interaction between GM1 and Aβ, and that an elevated cholesterol level in these domains could enhance Aβ aggregation at physiological concentrations. In support of this theory, Aβ was reported to be present in lipid rafts of mouse brains. These findings also explain the presence of GM1/Aβ in the brains of patients with AD who exhibit early pathological changes at the molecular level *(68)*. As mentioned previously, alterations in the cholesterol level in lipid domains, in contrast to alterations in the total cholesterol level, are suggested as potential causes of AD. Recent studies have also shown that the alterations in transbilayer cholesterol distribution—but not those in the total cholesterol level—are similar in synaptic plasma membranes of aged mice and mice that express human apoE4, as compared to those in the same membranes of younger mice and mice that express human apoE3 *(69,70)*. They showed that the largest changes occurred in the exofacial leaflet, where rafts as well as GM1 are believed to be located, suggesting that apoE is involved in the regulation of cholesterol distribution in rafts.

Concluding Remarks

As mentioned in the Introduction, many scientists agree that the cellular cholesterol level is involved in Aβ generation and that the prevalence of AD can be reduced by the treatment of patients with statins, which reduce cholesterol levels in serum and probably in CNS cells. The detailed mechanism underlying cholesterol-dependent modulation of Aβ synthesis and AD development is the next issue to be addressed. Determination of the association between statin treatment and inhibition of AD development and the establishment of statin therapy for AD and MCI are also important issues to be addressed.

Another perspective on the role of cholesterol in the formation of Aβ seeds is presented here. Although the concentration of Aβ in the extracellular space has not been determined, it is widely believed that Aβ concentrations in CSF are too low to form aggregates. Thus, it is important to elucidate the mechanism by which Aβ forms oligomers and amyloid. In this regard, it is feasible to theorize that Aβ seeds promote oligomerization and amyloid formation. Recent studies have demonstrated that cholesterol in the membrane is essential for formation of seeding Aβ and GM1 bound Aβ.

In addition to these important perspectives, a novel view of the putative role of cholesterol in the amyloid cascade is also proposed in this article. The findings in support of this theory show that oligomeric Aβ affects cellular cholesterol metabolism, leading to reduction of the cellular cholesterol level, which may induce AD-related pathologies. Based on these findings, it is possible that the role of cholesterol in AD pathogenesis is dualistic. Thus, a decreased level of cellular cholesterol may prevent AD development, yet may enhance AD pathologies when AD and MCI have already developed.

However, since these results are derived from basic research and no animal data or clinical data supporting this notion are currently available, further studies are required to determine whether decreased levels of cellular cholesterol promote AD pathologies in vivo.

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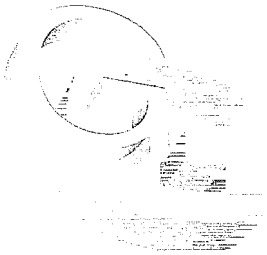
特　集　アルツハイマー病における新しい治療の試み



Aβとコレステロール代謝からみたスタチン系薬物による治療

道川　誠，柳澤勝彦

抄　録

アルツハイマー病発症機構とコレステロールの関係に関心が寄せられているのは，アポリポタンパクE4が強力な危険因子であること，疫学研究によって高コレステロール血症が危険因子であること，コレステロール降下薬（スタチン）服用が予防効果をもつこと，等が示されたためである．しかし，この両者の関連およびメカニズムについては依然不明な点が残っている．本当にスタチン服用によるコレステロール代謝調節によってアルツハイマー病発症を抑制できるのか，その理由はなぜか．社会的関心も影響も大きいだけに早急に結論をだすべき課題である．

Key words：アルツハイマー病，高コレステロール血症，スタチン，HDLコレステロール，アミロイドカスケード，アミロイドβタンパク

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はじめに

コレステロールを含む脂質代謝研究はおもに，血管内皮細胞，線維芽細胞，肝細胞，各種細胞株など非神経系細胞を用いて行われてきた．こうした研究の歴史は長く，その知見の集積は膨大である．しかし，最もコレステロールに富む臓器である脳（中枢神経系）におけるコレステロール代謝についての知見はきわめて少ない（あるいは少なかった）．そもそも，神経細胞は，その形態が他の細胞と異なる．神経突起の膜の表面積は細胞体のそれの数十倍から数百倍に及ぶこと[3]から，すべてのコレステロールを細胞体から末端まで運んでいたのでは早い変化（たとえばシナプス可塑性の維持や外傷後の修復など）に対応できないと考えられる．突起末端こそ，まさにシナプス可塑性を維持する場所であり，最近の研究によれば24時間以内に全シナプスの20%以上がturn overするほど激しく変化するとされるからである[36]．したがって，神経突起末端での膜の変化の維持には，細胞体からのコレステロール供給以外に，末端局所でのコレステロール代謝機構の果たす役割が大きいと考えられる．実際，細胞外液中のHDLコレステロールがシナプス可塑性維持に重要な役割を果たすことが示されている[29]．

近年，アルツハイマー病（Alzheimer's disease；AD）発症機構とコレステロールの関係に関心が寄せられ，脳内のコレステロール代謝に注目が集まっている．しかし，中枢神経系と体循環系は血液-脳関門によって隔絶され，中枢神経系には独立したコレステロール代謝系が想定されているため体循環系のコレステロール代謝の知見をそのまま脳内のそれとして援用することはできない．実際，中枢神経系（髄液中）にはLDL，VLDLなどのリポタンパク質とそれらに関連する多くのアポリポタンパク質は存在せずHDLのみが存在する．中枢神経系にHDLしか存在しないとなると，コ

Makoto Michikawa, Katsuhiko Yanagisawa：国立長寿医療研究センター痴呆疾患研究部
〒474-8522　愛知県大府市森岡町源吾36-3

レステロールを細胞から引き抜くだけとなってしまい，はたして脳内脂質代謝の恒常性は保てるのであろうか．こうしたきわめて基本的な疑問に答えることから筆者らの研究は始まった．

まず，同じHDLといっても，それを形成するアポリポタンパクが異なる点に注意すべきであろう．血液中ではアポリポタンパクAI（apolipoprotein AI；apoAI）がHDL形成に重要な役割を果たすが，中枢神経系ではアポリポタンパクE（apolipoprotein E；apoE）の役割が重要となる．なぜなら，apoAI受容体としてスカベンジャー受容体（SR-B1）やABCA1（ATP-binding cassette transporter A1）などが知られるが，末梢細胞がapoAI-HDLをこうした受容体を介して取り込み，LDLのようにコレステロール供給源として利用することはなく，いくつかのステップを介して肝細胞へと逆行輸送されると考えられる（もし途中で取り込まれればいわゆる“善玉コレステロール”としてのHDLの役割は意味を失う）．しかし，apoE-HDLの場合は話が違う．神経細胞，アストロサイト，ミクログリアおよびオリゴデンドログリアにはいずれも複数のapoE受容体が存在し，apoE-HDL複合体は体循環系のLDLのように，脂質供給作用をもつと考えられるからである．筆者らは中枢神経系内ではHDLがコレステロールの搬出と搬入の双方の役割を担っていることを裏づける研究結果を得ている（論文準備中）．

いずれにしても，apoEの対立遺伝子$\varepsilon 4$がAD発症の危険因子であるとするStrittmatterら[44]の発見は，AD発症機構における脂質の関与を強く示唆し，AD発症機構における脂質研究にその論理的根拠を与えた．また，最近の疫学研究によってコレステロールとADとの関連が示されるに及んで，両者の関連に焦点をあてる研究に関心が集まり，両者の関係が分子レベルで議論されるまでに至った．

各　論

1．血清コレステロール値とアルツハイマー病

Notkolaら[35]は1959～1974年間の患者データを解析し，血清コレステロール値と1989年時点でのAD発症の関連について検討した．その結果，①AD発症と発症前の長期間にわたる高コレステロール血症との間に有意な相関が存在すること，②AD発症前に血清コレステロール値が低下することを報告した．これは以前の報告[19]を支持し，Evansら[4]の横断的な研究もこの両者の関連を確認した．こうした結果から，比較的長期にわたり先行する高コレステロール血症の病歴がAD発症の危険因子であると結論されている．先行する高コレステロール血症の存在はADのみならずmild cognitive impairment（MCI）発症との間にも有意な相関があることが報告された[24]．しかし，この両者の関連を確定するためには縦断疫学調査検討による確認が必要である．

2．血清コレステロール値とapoE

さて，上記の関連が本当であれば，血清コレステロール値はapoEの遺伝子多型と関連して変化するのであろうか．この両者の関係については，すでに冠動脈疾患との関連からなされた多くの論文がある．最近の疫学調査結果でも，apoE2＜apoE3＜apoE4の順で血清コレステロール値が高いことが示されている[10]．他の報告も同様の結果を示しており，血清コレステロール値の高値という点からAD発症とapoE4の相関が見事に一致することを示している．以上の事実をまとめると，apoE4はapoE2やapoE3に比べて血清コレステロール値を上昇させることでAD発症促進にかかわっていると考えることができる．

3．コレステロールパラドックス；高総コレステロールあるいは低HDL-コレステロール：どちらが真犯人か？

それでは，上記の考え方で，矛盾はなく説明可能なのだろうか．実は，そう単純ではないかもしれない．なぜなら，血清中の高コレステロール値

あるいは高コレステロール食は，髄液中のコレステロール濃度に影響しない[5,9,21]とされるからである．かりに，血清中のコレステロール値が高いのが問題とするならば，なぜ脳内コレステロール代謝に影響しない高コレステロール血症でADが発症しやすくなるのか，そのメカニズムを単なる髄液のコレステロール濃度変化からでは説明ができないことになる．筆者らは，同じデータに対する別の解釈の可能性も考えている．たしかに，apoEのアイソフォーム依存的に血清中の総コレステロール値（あるいはLDLコレステロール値）は，apoE4＞apoE3＞apoE2の順に高い[1,10,22]．しかし，同時に，血清HDL値は逆にapoE2＞apoE3＞apoE4の順であることも示されている[1,10]のである．このHDLコレステロール値におけるapoEのアイソフォーム依存性は，おそらく筆者らが報告したようにapoEの逆行性コレステロール運搬作用（コレステロール搬出によるHDL新生作用）におけるapoEのアイソフォーム依存性で説明できるであろう[11,32]．いずれにしても，コレステロール値とapoEのアイソフォーム依存性を論じる場合には，着目すべきリポタンパクの種類（LDLかHDLか）によって逆の順番になるのである．これらの結果からいえば，「先行する高コレステロール血症がADの危険因子である」とする仮説は，「低HDLコレステロール値がADの危険因子である」とする仮説に理論上置き換え可能である．Notkolaらの調査でHDLコレステロールとの関連を調べ直してみればただちにわかることである．

では，なぜ総（あるいはLDL）コレステロールではなくHDLコレステロールを重要視するのか．その理由は，中枢神経系（髄液中）にはHDLコレステロールしか存在しない[5]からである．中枢神経系内のHDL新生はapoEによる脂質搬出機構に大きく依存している．したがって，血清HDLコレステロール値を前提とすれば，中枢神経系の細胞外液中（あるいは髄液中）のコレステロール量は，apoE2＞apoE3＞apoE4である可能性が高い．なぜなら，アストロサイトにおけるapoEによるHDL新生能はapoE2＞apoE3＞apoE4であるからである[11,32]．上記に加えて，HDLに着目すべき理由がある．前述したように，血清中の高コレステロール値あるいは高コレステロール食は，髄液中のコレステロール濃度に影響しない[5,9,21]．しかし，興味深いことに血清中のHDLコレステロール値は髄液中のコレステロール値（HDLと考えてよい）とよく相関する[5]とされる．これらは，血清HDLコレステロール値の低下，そしておそらくHDL形成機序から考えて脳内HDLコレステロール量の低下が，AD発症の危険因子になるとすれば，血清コレステロール値と髄液コレステロール値の関連によって説明がつくことを示している．実際，AD患者の血清HDLコレステロール値が低いとする報告[17,30]がある．AD患者髄液中のコレステロール代謝の詳細な検討は今後に待たなければならないが，すでに，AD患者髄液中のコレステロールを含む脂質濃度は対照群に比して低いとする報告[34]もある．以上から，神経細胞内コレステロール値が高いのがリスクだと簡単に考えることには慎重でなければならないと筆者らは考える．しかし，現在までの血清コレステロール値とAD発症に関する多くの研究結果の解釈に，この点を考慮する議論は欠落している．今後，髄液中の脂質解析がapoEアイソフォームとの関連でなされる必要があると思われる．

4．スタチンとアルツハイマー病

さて，長期にわたって先行する高コレステロール血症（あるいは低HDL血症）とADおよびMCIの発症率とに正の相関があるとすれば，当然ながらコレステロール降下薬（スタチン〈statin〉）はADおよびMCI発症率をさげるのではないか，という疑問が起こる．Wolozinら[47]は，コレステロール降下薬として知られているHMG-CoA reductase阻害薬（スタチン）の服用者では，非服用者あるいは他の薬物の服用者に比べてAD発症率の有意な低下がみられるという研究結果を発表した．また，Jickら[20]は，調査時にスタチンを服用して

いる群では，服用していない群に比べて痴呆疾患に罹患する頻度が低いと報告した．しかし，スタチンによるAD発症抑制の機序については，いまのところ不明である．

たしかに，細胞内コレステロール量とアミロイドβタンパク（amyloid β-protein；Aβ）産生の関連を明らかにした研究がなされ注目を集めている．すなわち，細胞内のコレステロール量を低下させると，アミロイド前駆体タンパク（amyloid precursor protein；APP）量には影響を与えずにAβ産生を低下させるというものである[41]．この結果は，細胞膜のコレステロール濃度をさげるとα-セクレターゼ活性を増強させ，αAPP量を増加させる一方，Aβ産生量を減少させるという報告によっても支持された[26]．さらに，大量のスタチン服用により血清コレステロール値が著減し，髄液中のAβ量が減少することがモルモットを用いた実験によって示された[8]．以上から，細胞膜のコレステロール濃度の上昇が直接Aβ産生量を増加させAD発症を促進させるが，スタチン服用による細胞膜のコレステロール濃度の低下はAβ産生量を抑制し，AD発症を抑制する可能性を示していると考えられた．しかし，これですべてを説明できるのであろうか．

Wolozinらの疫学研究によって検討されたスタチンはロバスタチン（lovastatin），シンバスタチン（simvastatin），プラバスタチン（pravastatin）の3種類であった．プラバスタチンが最もhydrophilicであるにもかかわらず，ADの発症率をさげたのはプラバスタチンとロバスタチンであった．こうしたスタチンは血液-脳関門を通過しにくいと考えられている．一方シンバスタチンはコレステロール値降下作用を発揮しているにもかかわらず，ADの発症を抑制しなかった．以上の結果は，スタチンによるAD抑制作用がかりにあったとしても，それが少なくとも脳内のコレステロール降下作用によるものかどうか疑問を残す．最近，常用量のスタチン服用者の髄液の解析によると，シンバスタチンを服用した人では，血清コレステロール値の低下に伴って脳内のコレステロール量の低下をきたすことが示されている[28]．しかし，スタチンの常用量では髄液のコレステロール量をさげるもののAβ産生には影響しないとする報告[9]があり，スタチンのAD抑制効果とAβ産生との関連には否定的である．スタチンによって髄液中のAβ量の低下を招いたとする研究[8]は，通常服用量の100倍も高いスタチン量を投与したためであり，疫学研究でみられた抑制効果がAβ量の低下によるものとは考えられないとしている．こうした混乱は*in vivo*マウス実験でもみられる．すなわち，高コレステロール食により脳内Aβ沈着が亢進し，亢進の程度は血清および髄液コレステロール濃度に比例する[37,42]という報告がある一方，高コレステロール食により脳内Aβ量が低下するとするものもあり，これらの一貫した説明がでない状況である[18]．餌の脂質構成があまりにも極端である，あるいは動物種や遺伝子操作による影響などがあるのかもしれない．

以上のような理由から，スタチンのもつコレステロール合成抑制作用以外の作用による可能性も当然検討されなければならないだろう．実際，スタチンはコレステロール合成阻害以外に，細胞内シグナル伝達や細胞増殖に関与するGタンパクの修飾に必要な中間産物であるfarnesyl pyrophosphateやgeranylgeranyl pyrophosphateなどの産生を阻害するほか，endothelial nitric oxide synthase（eNOS），inducible NOS（iNOS）やサイトカインなどの産生を抑制し，脳内炎症を抑制することが知られている[14]．また，動脈硬化がADおよび血管性痴呆両者の危険因子であるとの報告[15]があることから，高コレステロール血症は直接的には動脈硬化促進を介してADの危険因子となっている可能性，そしてスタチンは炎症性疾患でもある動脈硬化を抑制すること[38,43]でAD発症を抑制している可能性がある．

5．コレステロールとアミロイドカスケード

これまで，ADとコレステロールの関連をその発症機構との関連から述べてきたが，本項ではこ

の両者の関連を別の角度から考えてみたい．Aβは，その凝集状態に依存して生物活性を発揮するとされ，アミロイドまたは線維化Aβが神経細胞におけるタウ（τ）のリン酸化の促進および神経細胞死を誘導するとされてきた．しかし最近，生理的に存在するアミロイドになるまえのオリゴマーAβが神経の機能障害[46]や細胞死等[13,45]を引き起こすことが示されるに及んで，アミロイド形成前のオリゴマーAβこそがAD発症メカニズムの主体を担うのではないかとの考え方が提起されるようになった[23,25]．以下に紹介する筆者らの研究も，この考え方を支持し発展させた．

従来から，髄液中に存在するHDLにAβが結合していることが報告されてきた．HDLにはapoEも含まれることからapoE受容体を介してHDL複合体を取り込むことによってAβが除去されるのではないかとする仮説とそれを検証する一連の研究がある[16]．しかしHDL複合体の形成過程および生（病）理学的意味については十分に理解されているわけではなかった．筆者らは，Aβの神経細胞内コレステロール代謝に対する影響の解析をとおして，HDL複合体形成過程を明らかにした．すなわち，①オリゴマーAβが神経細胞膜よりコレステロール，リン脂質およびGM1ガングリオシド等を引き抜き（搬出し）HDL様粒子を形成するが，この脂質-Aβ複合体はapoEによって産生させるHDL様粒子と異なり，細胞に取り込まれないこと[33]，②オリゴマーAβは神経細胞内コレステロール合成を抑制し，最終的にその量を減少させる働きがあること[12]である．こうした作用は単体Aβにはみられず，むしろ抗活性酸素作用を発揮し細胞保護的に働いた[48]．AD脳ではオリゴマーAβ量が増加すると考えられることから，ADでは，増加したオリゴマーAβが神経細胞内コレステロール代謝を変動させている可能性が示唆される．

筆者らをはじめとするいくつかのグループの研究結果を総合すると，以下のような考え方が可能になる．すなわち，オリゴマーAβが細胞内コレステロールを減少させ[12,33]，コレステロール量の減少がタウのリン酸化亢進[6,27]，シナプス可塑性および機能の低下[7,27,29]，そして神経細胞に特異的な細胞死の誘導[31]等のAD病理に類似した諸現象を招くということである．こうしたオリゴマーAβによって影響される細胞内コレステロール代謝の恒常性をapoEはHDLの取り込みおよび搬出作用によって維持していると考えられる．しかし，筆者らの示したようにapoEのHDL形成作用がアイソフォーム依存的である[11,32]ことから，apoEはコレステロール代謝の恒常性維持能力の違いをとおしてAD発症機構に関与しているのではないだろうか．

コレステロール欠乏とタウのリン酸化亢進との関連については，さらにコレステロール代謝異常を中核病態とするNiemann-Pick disease, type C（NPC）のモデルマウス脳で解析され，MAPK（mitogen-activated protein kinase）活性の上昇およびタウのリン酸化亢進[39]，cdk5（cyclin-dependent kinase-5）の活性化亢進や他の細胞骨格タンパクのリン酸化亢進が確かめられている[2]．これらの機序として，NPC1欠損細胞では，マイクロドメインを含むdetergent-insoluble membrane fraction中のコレステロールの低下が，マイクロドメインの構造および機能の障害を招き，それが細胞内シグナルの異常を誘導している可能性[40]を考えている．以上をまとめると，アミロイドカスケードにおいては，コレステロール量はむしろ低すぎないことが大事である[12]と考えられる．もちろん，これらは*in vitro*または動物モデル上での知見であり，当然ながらただちにヒトに適用できるわけではないが，少なくともAD発症後のスタチン服用には注意が必要かもしれない．

文　献

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