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Alzheimer's Disease, Cholesterol, and Statins: The Junctions of Important Metabolic Pathways

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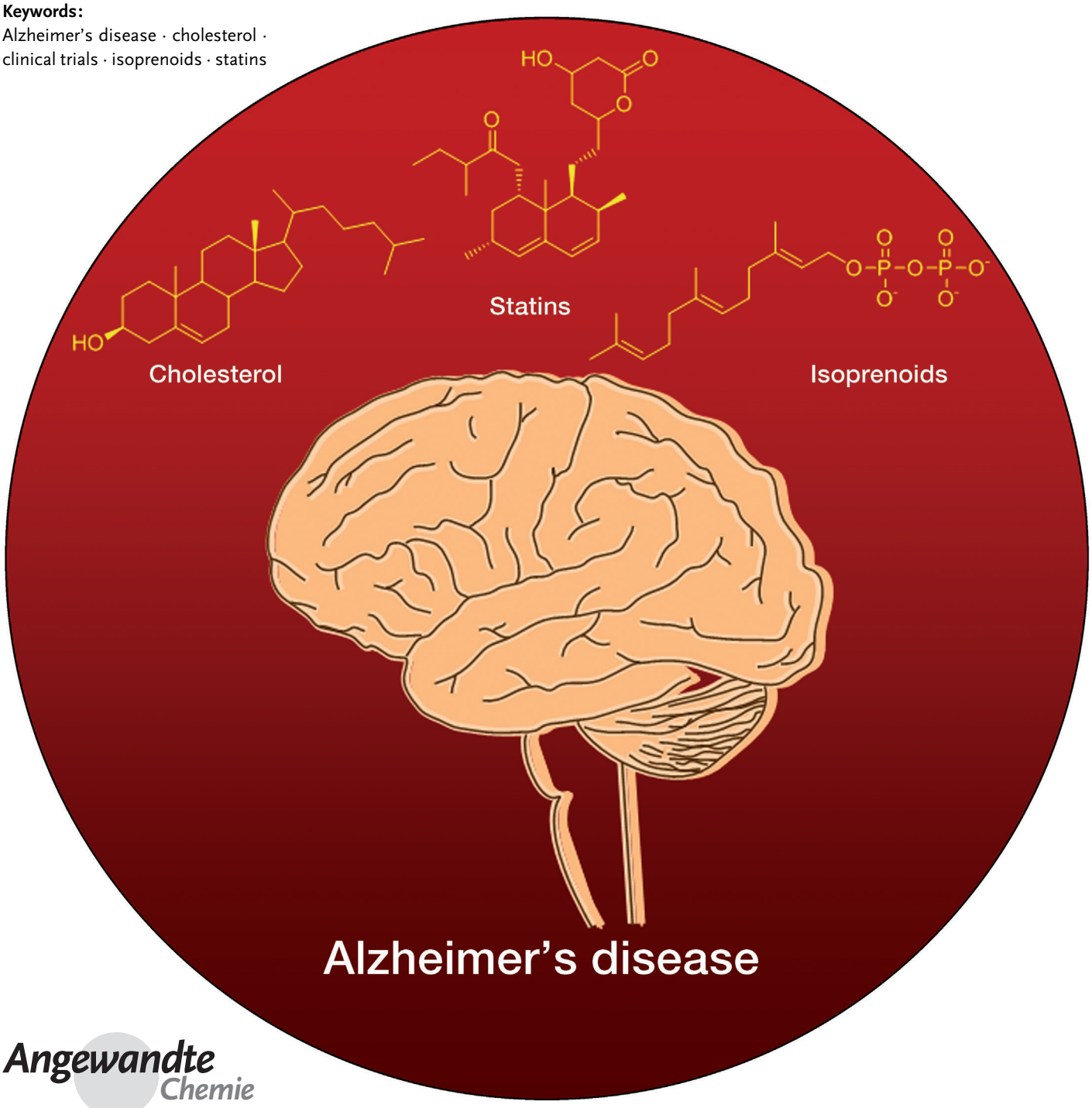

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Recent years have seen a significant increase in published data supporting the positive effects of statins on neurodegenerative diseases, in particular on Alzheimer's disease. Statins show neuroprotective activity by a combination of different cellular and systemic mechanisms that are based on the inhibition of the biosynthesis of cholesterol and isoprenoid by-products. The promising results obtained in vivo and in epidemiological studies are generally not in accordance with those of placebo-controlled randomized clinical trials. Nevertheless, these results make statins valuable assets for disease prevention rather than therapeutic agents for use when disease symptoms are already displayed. Thus, the modulation of midlife cholesterol and/or statin administration prior to the appearance of dementia or cognitive impairment may have a better long-term outcome.

From the Contents

1. Introduction

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) redutase, commonly known as statins (Figure 1), are extensively prescribed drugs in the pharmacological treatment of hypercholesterolemia and dyslipidemia.[1] These drugs reversibly and competitively inhibit the rate-limiting step of the mevalonate pathway and thus block the de novo synthesis of cholesterol and isoprenoid by-products (Figure 2). It is generally accepted that statins display a vast range of biological activities that are unrelated to their cholesterol-lowering effect. These pleiotropic effects of statins are far too immediate and variable to be explained only in terms of their long-term effect on atherogenesis.[2] This wide activity range has offered new perspectives on statin pharmacology and with them a window of new potential therapeutic applications. Statin pleiotropy is particularly relevant in neuroprotection and neurodegenerative disorders, such as Alzheimer's disease (AD). Herein, we review the biochemical connections between statins and Alzheimer's disease with a focus on the pathological pathways that link cholesterol, isoprenoids, and apolipoprotein E (ApoE) with neuronal damage and neurodegeneration.

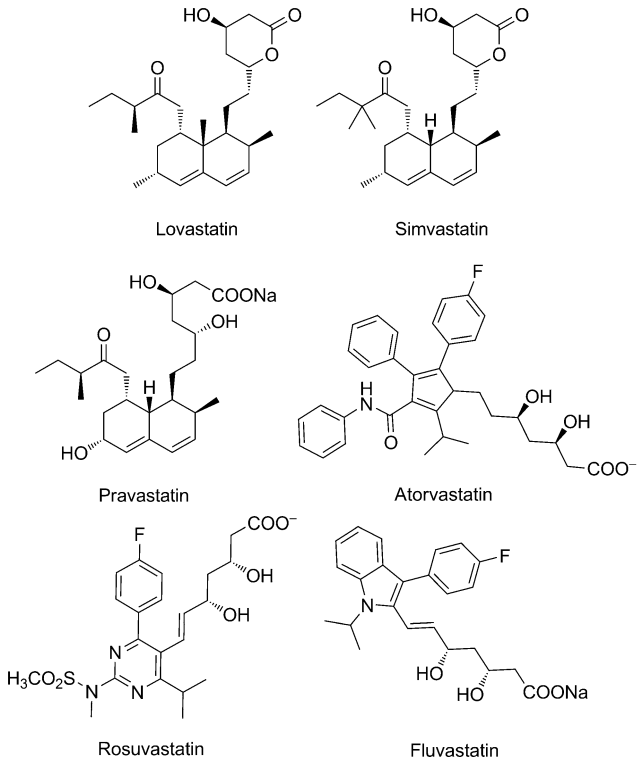

Figure 1. Chemical structures of the most commonly prescribed statins.

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2. Statins

Statins (Figure 1) can be categorized according to their origin, plasmatic half-life, physicochemical properties and specific activity. Lovastatin, simvastatin, and pravastatin are obtained by fungal fermentation, as opposed to synthetic rosuvastatin, atorvastatin, and fluvastatin. All statins undergo high first-pass metabolism by the liver. Most of the absorbed dose is excreted in the bile, and a small fraction in the urine. The plasma half-lives of these drugs range from 1 to 3 h, except for those of atorvastatin (14 h) and rosuvastatin (19 h). Pravastatin and rosuvastatin are hydrophilic, lovastatin, simvastatin, and atorvastatin are hydrophobic, and fluvastatin has intermediate characteristics. Lovastatin and simvastatin are administered as inactive lactone prodrugs, which are hydrolyzed in the gastrointestinal tract to the active β-hydroxy derivatives, whereas pravastatin and fluorine-containing atorvastatin, fluvastatin, and rosuvastatin are administered as active compounds.[3]

3. Alzheimer's Disease

3.1. General Overview

Alzheimer's disease (AD), a complex heterogeneous neurodegenerative disorder, is the most common cause of dementia in the elderly and the single greatest source of dysfunction among persons over age 85.[4] In 2000, there were 25 million persons afflicted with AD worldwide (4.5 million in the USA), a number expected to increase to 114 million by 2050 (13.2 million in the USA).[5] In Europe, 7.3 million citizens suffer from dementia, and every year 1.4 million new cases occur. The European Union predicts a twofold increase in these numbers by 2040 (http://www.alzheimer-europe.org/). Current therapeutic alternatives, such as acetylcholinesterase inhibitors (AChEIs), are unable to prevent disease progression. In line with these predictions, an effective cure for AD remains to be discovered, and new drugs are unlikely to come onto the market until 2020;[6] thus, new effective drugs for AD are required to meet a pressing clinical need.

3.2. Symptoms and Risk Factors

AD patients gradually develop insidious cognitive deficits that become incapacitating in the advanced stage of the disease. Characteristic symptoms include memory alterations, disorientation, aphasia, judgment and performance disorders, and personality changes. These debilitating symptoms severely decrease the quality of life of patients and lead to complete dependence and, inevitably, hospitalization.[7] The most common form of Alzheimer's disease, accounting for approximately 95% of AD cases, is the late-onset AD (LOAD) form, in which symptoms generally start to appear at around age 65. The major risk factors for LOAD are advanced age and the presence of the allele ε4 of the apolipoprotein E (*APOE*) gene. Only a small percentage of AD cases (5%) can be described as familial AD, the early-onset form of the disease (EOAD), in which symptoms appear earlier in life.[7] Familial AD risk is markedly genetic and associated with mutations in the presenilin-1 (*PSEN1*), presenilin-2 (*PSEN2*), and amyloid precursor protein (*APP*) genes.[8a] The *APP* gene is encoded in chromosome 21. Mutations in this gene are associated in particular with Down syndrome (DS).[8a] With the exception of a single reported case, all subjects with DS demonstrated triplication of the APP gene locus (21q21).[8b]

Tiago Silva received his MSc in Pharmaceutical Sciences from the University of Porto, Portugal, where he is currently pursuing PhD studies in Pharmaceutical Sciences (toxicology) under the supervision of Professor Fernanda Borges. His research is focused on the development of multitarget drugs for neurodegenerative diseases.

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José Teixeira graduated in Biochemistry from the University of Trás-os-Montes and Alto Douro in Portugal. He obtained his MSc in Biochemistry from the University of Porto, where he is currently pursuing PhD studies in Pharmaceutical Sciences under the supervision of Professor Fernanda Borges. His research is focused on the development of mitochondria-targeted antioxidants based on a cinnamic acid scaffold as a therapeutic solution for neurodegenerative diseases.

Fernando Remião is Associate Professor in the Department of Biological Sciences of the Faculty of Pharmacy at the University of Porto and senior researcher at associate laboratory REQUIMTE. He received his PhD in Pharmaceutical Sciences (toxicology) from the University of Porto. His current research is focused on toxicokinetics, in particular the toxicological consequences of MRD and MRP activity and expression modulation and on the cardiotoxicity of mitoxantrone.

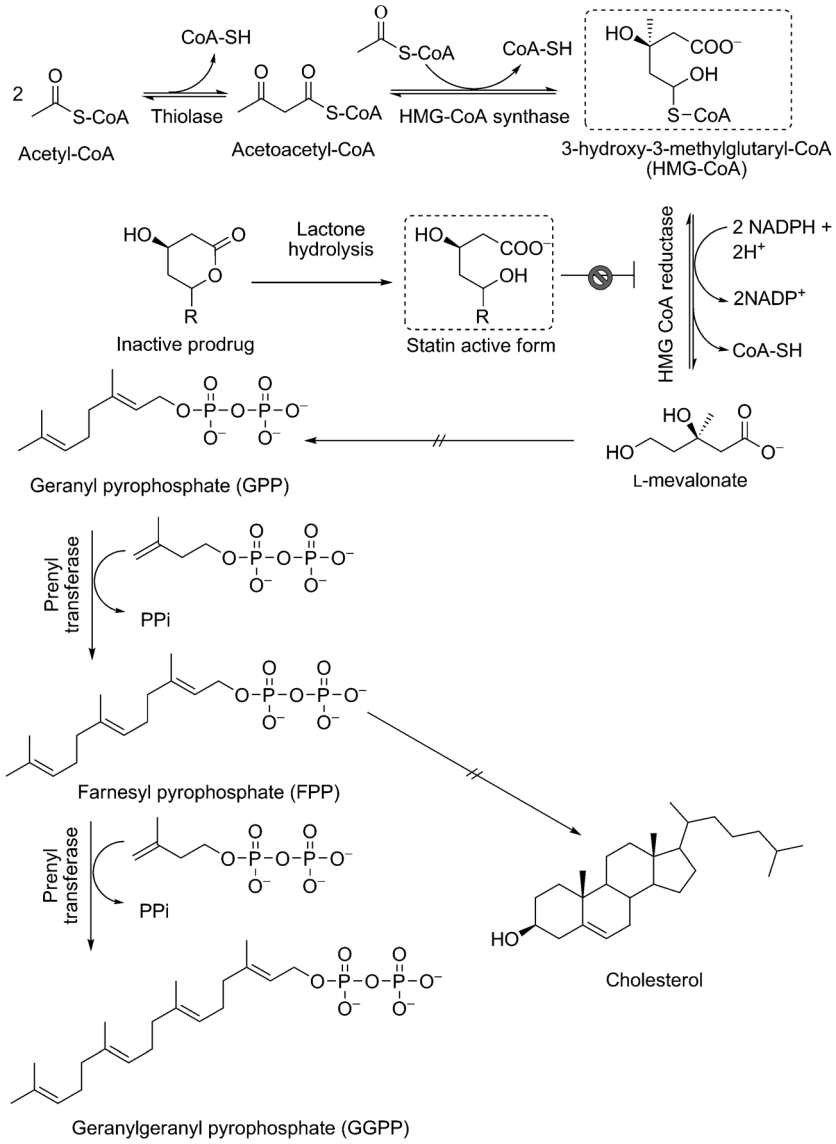

Figure 2. The mevalonate pathway and the mechanism of action of statins. The active forms of the redutase inhibitors (statins) are structural analogues of the intermediate that is formed between the enzyme and the substrate in the rate-limiting step of the mevalonate pathway (3-hydroxy-3-methylglutaryl-CoA). These analogues cause partial inhibition of the enzyme and thus disrupt not only the synthesis of cholesterol, but also that of by-products known as isoprenoid intermediates. PPi = pyrophosphate.

3.3. β-Amyloid, Tau Protein, and Protein Misfolding

The etiology of AD is a complex process resulting from a combination of genetic and neurobiological factors and is often compared with an incomplete puzzle: the Alzheimer's puzzle.[7,9] The neuropathological hallmarks found in the brains of AD patients are neuronal loss in regions related to memory and cognition, neurotransmitter depletion (particularly acetylcholine), and synaptic alterations.[7,9] Microscopically, the most common findings are abnormal protein deposits, including senile neuritic plaques (SNPs) and neurofibrillary tangles (NFTs).[10] Senile plaques are the result of the extracellular accumulation of insoluble aggregates of β-amyloid protein (Aβ), whereas NFTs occur intracellularly and are composed of paired helical filaments of hyperphosphorylated tau protein. These abnormalities lead to the activation of neurotoxic cascades and to cytoskeletal changes that eventually cause synaptic dysfunction and neuronal death.[10] Protein misfolding and abnormal aggregation play a critical role in AD pathology, as they lead to the formation of insoluble pathological conformers that cause neuronal degeneration and cellular death.[10b] The main feature of AD etiology is the multiplicity of pathological stimuli associated with increased risk of disease development and progression. Possible mechanisms by which these stimuli promote AD are commonly referred to as hypotheses. Indeed, several such hypotheses have been proposed to date, including the amyloid hypothesis,[10] the cholinergic hypothesis,[11] the glutamatergic hypothesis,[11c,12] the oxidative-stress hypothesis,[13] the metal hypothesis,[14] and the inflammatory hypothesis.[15]

β-Amyloid protein is formed after the cleavage of APP (Figure 3a) by proteolytic enzymes known as secretases. The enzymes involved have been identified as β-, α-, and γ-secretase. APP can undergo two distinct processing pathways with different outcomes (Figure 3b). The nonamyloidogenic pathway yields nontoxic soluble products (α-sAPP and the P3 fragment), whereas the amyloidogenic pathway, which proceeds through an initial cleavage by β-secretase, results in the formation of nonsoluble and toxic Aβ.[16] In many instances, an increase in nonamyloidogenic APP metabolism is coupled to a reciprocal decrease in the amyloidogenic processing pathway, and vice versa, as the α- and β-secretases compete for the same substrate.[16] Although Aβ is produced by normal cellular metabolism, mutations that lead to familial AD increase amyloidogenic APP processing. As cleavage by γ-secretase is not precise, the amyloidogenic pathway can produce different forms of Aβ: primarily $A\beta_{40}$ and $A\beta_{42}$, which contain 40 and 42 amino acids, respectively. $A\beta_{42}$ has a greater tendency to aggregate and is therefore more toxic to neurons than $A\beta_{40}$. Furthermore, in AD, the $A\beta_{42}/A\beta_{40}$ ratio is increased.[17] Insoluble Aβ is deposited in neurons and forms aggregates that accumulate into SNPs. These histopathological features are not exclusive to AD. They are also found in the ageing process and in other types of dementia, but they seem to be found in larger amounts in AD patients, where they cause neurodegeneration, synaptic loss, and neuronal death.[17] Associated with an increase in amyloid production is

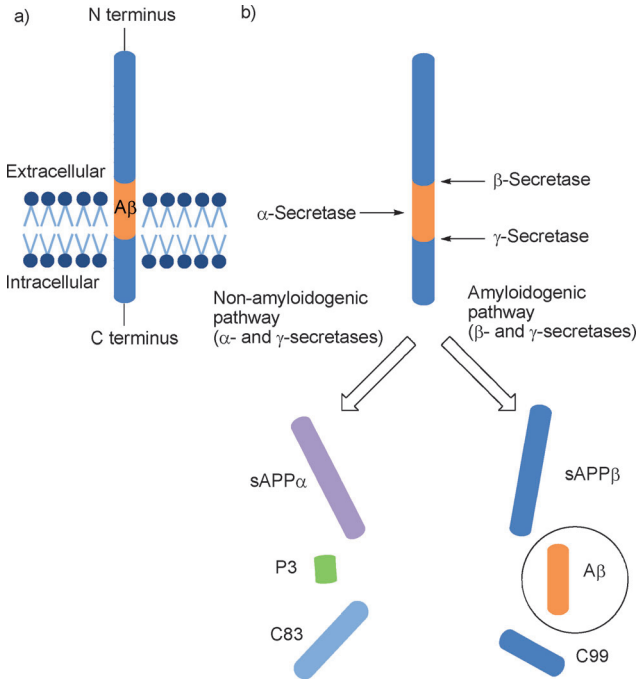

Figure 3. a) Schematic representation of the amyloid precursor protein (APP). APP is a transmembrane glycoprotein with synaptogenic and neurotrophic properties that is encoded in chromosome 21. b) Non-amyloidogenic (left) and amyloidogenic (right) cleavage of APP. The non-amyloidogenic pathway involves sequential cleavage by α- and γ-secretase to yield nontoxic soluble products (α-sAPP and the P3 fragment), whereas the amyloidogenic pathway occurs by initial β-secretase cleavage, which results in the formation of nonsoluble and toxic Aβ.[23] β-Secretase is also referred to as β-site APP cleaving enzyme I (BACE1), and γ-secretase is a complex formed from the proteins PSEN1 or PSEN2, nicastrin, Aph1, and presenilin enhancer 2 (Pen2). Three putative α-secretases have been identified: TNF-α converting enzyme (TACE; TNF = tumor necrosis factor) and desintegrin and metalloprotease domain proteins 9 and 10 (ADAM-9 and ADAM-10).[24]

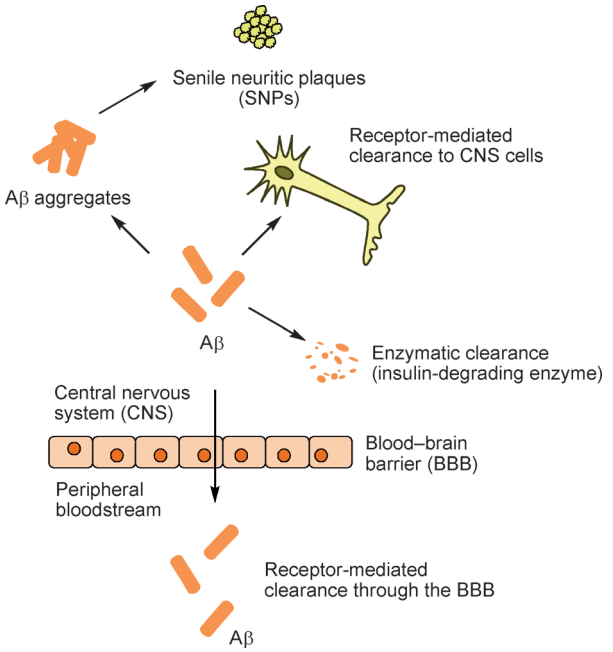

Figure 4. Amyloidogenesis versus Aβ clearance. Aβ can either aggregate or undergo receptor-mediated clearance through the blood–brain barrier (BBB) or into CNS cells. Aβ can also undergo proteolytic degradation by the insulin-degrading enzyme (IDE).[35] This equilibrium is deregulated in AD, and Aβ aggregation is favored over clearance. A net buildup of Aβ aggregates inside the CNS results and ultimately leads to disease progression.

also a decrease in both the receptor-mediated and the enzymatic clearance of Aβ[18] (Figure 4). The increased production and decreased clearance account for the overall amyloid buildup within the central nervous system (CNS). These findings support the amyloid hypothesis. Although Aβ is toxic to neurons in cell culture, the observation that Aβ deposits formed by APP overexpression in transgenic mice did not cause sufficient neuronal death suggests that additional factors are necessary to promote the progression of the disease.[19] Thus, AD is the outcome of a combination of systemic pathologic stimuli over an extended period of time.[20]

4. Statins, Hypercholesterolemia, and the Risk of Alzheimer's Disease

A lower prevalence of AD-like dementia in patients undergoing lipid-lowering therapy was reported in case-control retrospective cohorts[25] and in observational studies.[26] Furthermore, a statistically significant reverse relationship between statin use and AD was observed,[27] although contrary findings were also described in other large epidemiological studies.[28] Open trials with AD patients found both lower levels of biomarkers of the disease in the cerebrospinal fluid (CSF) and slight increases in the cognitive performance (on the Alzheimer's disease assessment scale: the ADAS-cog score) of patients undergoing statin therapy.[29] These findings were strongly corroborated by the results of the Rotterdam Study,[30] a prospective study involving 6992 subjects over a period of 9 years. Haag et al.[30] concluded that statin therapy reduced the risk of late-onset Alzheimer's disease (LOAD) by almost 50%.

Although controversial, the cholesterol hypothesis has received increasing attention over the last decade. In vivo studies showed that plasmatic cholesterol levels and amyloidogenesis are closely correlated.[31] According to these studies, an increase in dietary cholesterol intake boosts β-amyloid (Aβ) levels and causes extensive deposition of SNPs in the brain tissue. Both of these microscopic findings are classic biomarkers of AD. These findings are further supported by epidemiological studies, which associate hypercholesterolemia during middle age with a higher risk of dementia and AD,[32] although other findings seem to contradict this hypothesis.[33] The Hisayama Study[34] established that abnormal lipid metabolism and dyslipidemia increased the risk of plaque-associated pathology and AD.

5. Cholesterol and Alzheimer's Disease

Cholesterol is the main sterol in animal tissues. Its amphipathic structure is composed of 27 carbon atoms, a polar OH group at C3, and a large hydrophobic moiety (Figure 2). Cholesterol is not only a major component of eukaryotic membranes, but it also functions as a biosynthetic precursor of important bioactive molecules, such as steroid hormones and bile acids. The main sources are dietary intake and endogenous hepatic biosynthesis through the mevalonate pathway. A small amount of biosynthesized cholesterol is incorporated in the hepatocyte membranes, but the majority is exported as bile acids (BAs) or cholesterol esters (CEs). Cholesterol and CEs are hydrophobic and require special transporters called lipoproteins (Figure 5). Lipoproteins are macromolecular complexes of phospholipids and specific carrier proteins known as apolipoproteins, which surround a lipid core of cholesterol, CEs, and triglycerides (TGs). Lipoproteins can be categorized according to the amount of lipids in the core, the type of apolipoprotein, size, and density. Four types are commonly distinguished: HDLs (high-density lipoproteins), LDLs (low-density lipoproteins), VLDLs (very low density lipoproteins), and chylomicrons (Figure 5).[36]

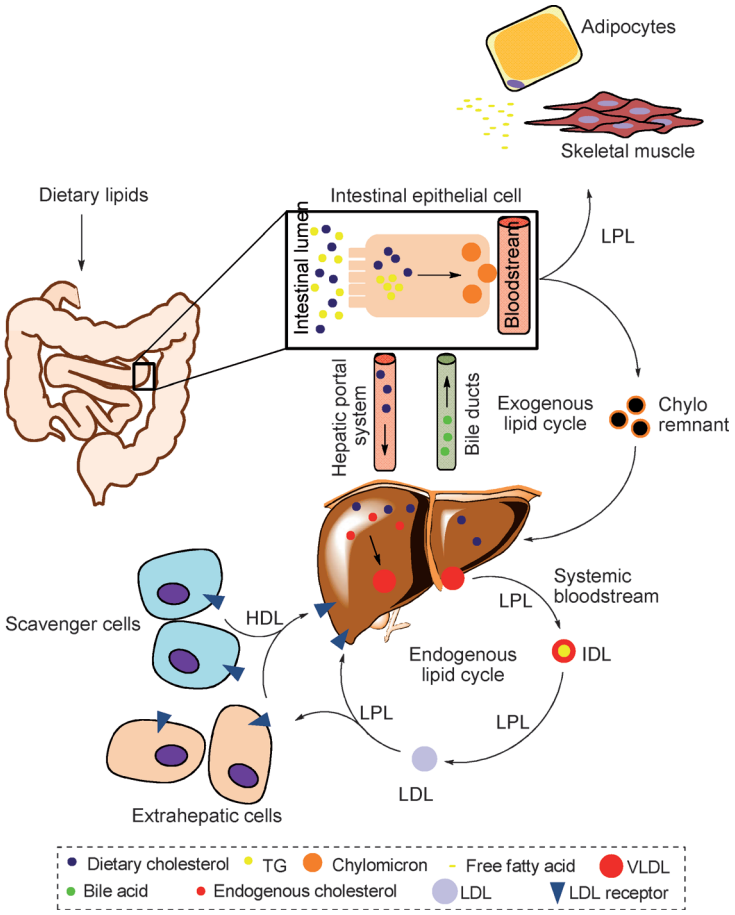

Figure 5. Lipoprotein metabolism and cholesterol transportation. The lipid content of the diet is incorporated into chylomicrons, which are mainly composed of triglycerides (TGs). The TGs are cleaved into glycerol and free fatty acids (FAs) by lipoprotein lipase (LPL) and deposited in muscle and adipose tissue. Remnant chylomicrons ("chylo remnant"), with proteins and cholesterol, are directed to the liver. Endogenous lipids and hepatic cholesterol are distributed in the muscle and adipose tissue by very low density lipoproteins (VLDLs). As the lipid content decreases, VLDLs turn into low-density lipoproteins (LDLs), which can either proceed with the extrahepatic delivery of cholesterol or be recaptured by the liver through LDL receptors. Excess cholesterol is brought back to the liver by high-density lipoproteins (HDLs) in a process known as reverse cholesterol transport.[37]

Cholesterol levels and the cellular distribution of cholesterol have a major influence on amyloidogenesis.[38] The amyloidogenic processing of APP takes place in small membrane-adjacent domains known as lipid rafts.[23,38] According to the definition stated at the Keystone Symposium of Lipid Rafts and Cell Function (2006), rafts are small heterogeneous domains (100–200 nm) enriched in steroids and sphingolipids, with a role in a wide range of cellular processes. The β- and γ-secretase enzymes, which are responsible for the amyloidogenic pathway, are located at the surface of these cholesterol-enriched sites.[16,23,38] In vitro and in vivo studies have shown that an increase in cholesterol levels enhances β- and γ-secretase activity and thus promotes APP metabolism by the amyloidogenic pathway (Figure 6). A decrease in intracellular cholesterol leads to structural rupture of the lipid rafts and thus favors α-secretase-catalyzed non-amyloidogenic APP cleavage, which leads to a significant decrease in Aβ levels.[23,38,39]

Cholesterol also plays a central role in atherosclerosis as a major component of atheroma plaques. Hypercholesterolemia is closely associated with the formation of atheroma plaques, which progressively reduce the patency of intracranial blood vessels and thus cause hypoperfusion and ischemic brain damage. Brain ischemia promotes APP expression and damages the blood–brain barrier (BBB); as a result, the clearance of cerebral Aβ is compromised. Observations in animal models suggest that ischemia enhances not only Aβ formation, but also SNP and NFT deposition (Figure 6).[40] Atherosclerosis is also the physiopathological cause of vascular dementia (VAD), which accounts for 15–20% of all cases of dementia and is thus the second most frequent form of dementia after AD.[41]

The deregulation of cholesterol homeostasis and metabolism is frequently observed in AD patients and is characterized by lowered HDL and increased LDL, (24*S*)-24-hydroxycholesterol, and 27-hydroxycholesterol levels in comparison with those of healthy control subjects.[42] (24*S*)-24-Hydroxycholesterol (24-S-OH-Chol), also known as cerebrosterol, is a hydrophilic cholesterol metabolite formed in the brain by the action of cholesterol 24*S*-hydroxylase. 24-S-OH-Chol is capable of crossing the BBB to the plasma (Figure 7), and hence can be used as a marker for central cholesterol elimination.[42b] Higher levels of this metabolite in the cerebrospinal fluid (CSF) suggest an increased cerebral cholesterol load, which, along with SNP and NFT deposits, is a common feature of the brains of AD patients in post-

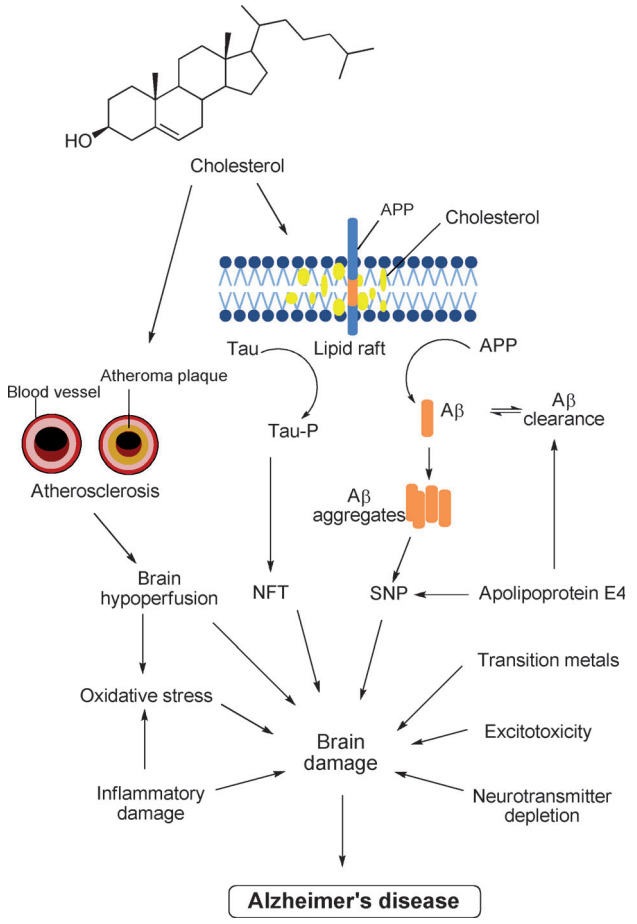

Figure 6. Role of cholesterol in neurodegeneration and Alzheimer's disease. Cholesterol facilitates amyloidogenesis by providing structural stability to membrane-adjacent lipid rafts, in which the proteolytic cleavage of APP to Aβ occurs. The accumulation of oxidized cholesterol in the walls of the blood vessels contributes to the formation of atheroma plaques. The reduction of cerebral blood flow and oxygen supply by these plaques is known to promote the release of deleterious oxidative species. Tau-P is hyperphosphorylated tau protein.[49]

mortem examinations.[39] Certain polymorphisms in the gene that encodes for cholesterol 24*S*-hydroxylase (the enzyme that metabolizes cholesterol into 24-S-OH-Chol) have been associated with a higher risk of dementia and AD.[8,42b] The other metabolite with an oxidized side chain, 27-hydroxycholesterol (27-OH-Chol), is formed systemically and is capable of crossing the BBB from the plasma to the CNS. The flux of 27-OH-Chol towards the brain is a key link between AD and hypercholesterolemia, since at high plasmatic levels, cholesterol can diffuse into the brain as 27-OH-Chol (Figure 7).[42b]

Further evidence for the deregulation of cholesterol metabolism in AD is the increased activity of acyl-CoA cholesterol acyltransferase (ACAT). As a result, more CEs are produced, and these CEs in turn promote amyloidogenesis.[42a] A possible pharmacological approach with ACAT as a potential drug target was suggested by in vitro studies with ACAT inhibitors, which led to the complete abolishment of amyloidogenesis.[38,43] In parallel with enhanced ACAT activity is an observed decrease in lecithin cholesterol acyltransferase (LCAT), a crucial enzyme in the reverse cholesterol-transport process by HDLs (Figure 7).[36] The combination of increased ACAT and decreased LCAT activity relative to that in healthy control subjects offers a plausible explanation for the elevated cholesterol levels observed in AD patients. In this context, Aβ is capable of modifying the cellular distribution of cholesterol as well as its esterification rate (free cholesterol/CE), as shown in Figure 6.[39] Furthermore, cholesterol can directly bind to APP and the C99 fragment (Figure 6), and this binding contributes to amyloidogenesis and AD. The association of APP/C99 with cholesterol may favor the partitioning of APP into membrane domains enriched in the proteases of the amyloidogenic pathway.[44]

6. Isoprenoids and Alzheimer's Disease

The long-chain isoprenoids dolichol and ubiquinone participate in membrane organization and mitochondrial oxygen consumption, respectively. The short-chain isoprenoids farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP; see Figure 2) bond covalently to small GTPases and are involved in the post-translational modification of small regulatory proteins by means of a process known as isoprenylation, which is essential for intracellular protein trafficking and membrane anchoring.[45] Isoprenylation targets are small proteins that act as molecular switches. When activated (connected with GTP), they control a wide range of intracellular signaling pathways and thus activate different types of cells.[45]

The isoprenylation of small GTPases by FPP and GGPP has attracted much attention owing to the potential pathological contribution of this process to diseases both within and outside the CNS. It is becoming increasingly recognized that isoprenylation is relevant both to ageing and to the physiopathology of AD. Isoprenylation is crucial for the activation of immune cells and immune response, both of which are key mediators of neuroinflammation (Figure 8). Not only do isoprenoid lipids appear to regulate the activities of α-, β-, and γ-secretase during APP metabolism, but isoprenoid-dependent processes have also been proposed in the modulation of glial activation, tau phosphorylation, and synaptic plasticity.[24,46] Eckert et al. (2009)[46] found that GGPP and FPP levels were significantly higher in brain tissue of AD patients (56 and 36 %, respectively) relative to those in normal control samples. Their results suggest that isoprenoid regulation may be altered in AD.

The activation of effectors downstream of prenylated small GTPases, such as the protein kinases ROCK (Rho-associated coiled–coil-forming protein kinase), has been implicated in APP metabolism. Furthermore, GGPP is known to stimulate the γ-secretase-mediated cleavage of APP and Aβ secretion, and to inhibit the reduction of Aβ production by the Rho/Rock pathway. The protein Rac 1 promotes the Aβ-induced generation of reactive oxygen species (ROS) and thus contributes to neuronal damage and to disease progression.[47] To define the importance of isoprenylation in AD progression, it was suggested that two pools of Aβ exist and appear to function independently of each other, whereby the intracellular pool is regulated by

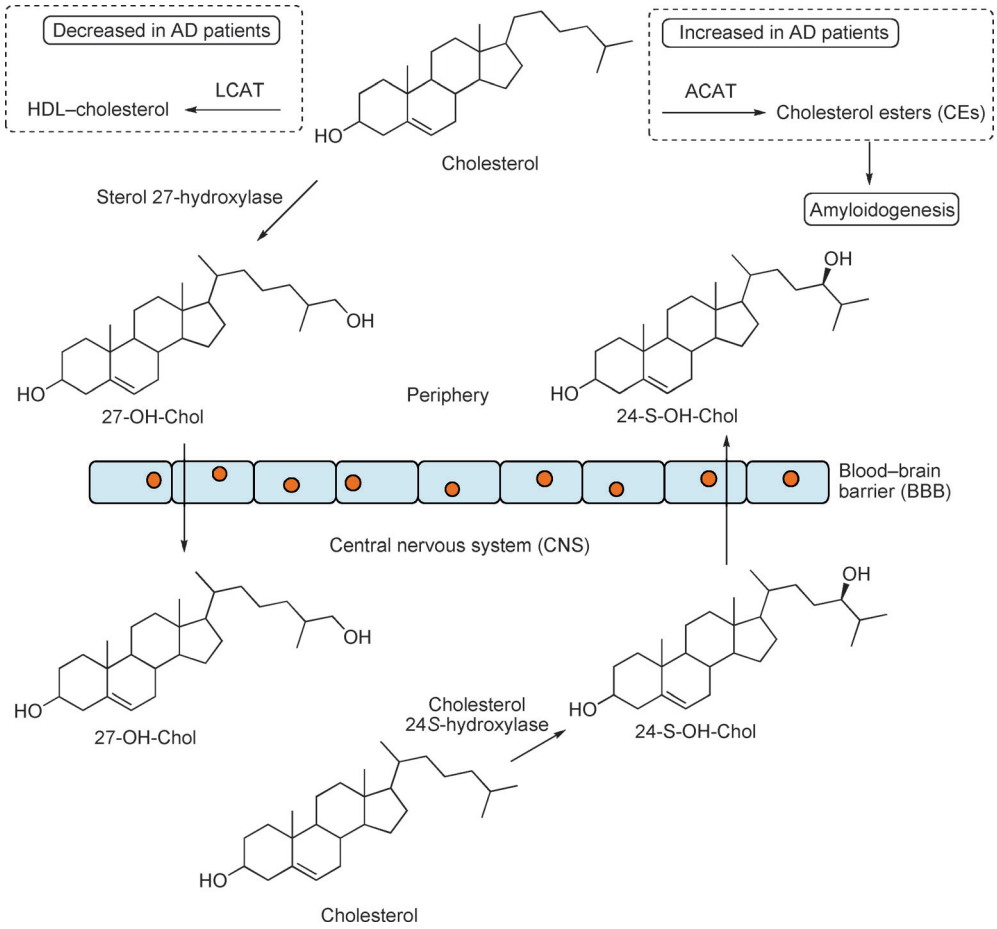

Figure 7. Cholesterol transportation between plasma and the central nervous system (CNS). Cholesterol efflux outward the CNS occurs via the hydroxylated metabolite 24-*S*-hydroxycholesterol (24-S-OH-Chol) that is able to cross the blood–brain barrier (BBB). The presence of this metabolite in plasma is considered a biomarker of an increased central cholesterol load. Furthermore, plasmatic cholesterol can be oxidized to 27-hydroxycholesterol (27-OH-Chol), a metabolite that is able to cross the BBB and reach the CNS, being a key link between hypercholesterolemia and increased cerebral cholesterol.

isoprenoids, and the secreted pool is regulated by cellular cholesterol levels.[48]

7. Apolipoprotein E and Alzheimer's Disease

Apolipoprotein E (APOE) is a 34 kDa protein with 299 amino acid residues and is the main cholesterol carrier in the brain. It is synthesized by astrocytes and, to a smaller extent, by microglia cells.[35] There are three alleles of the *APOE* gene: ε2, ε3, and ε4. The ε4 allele of the *APOE* gene is the main genetic risk factor for LOAD.[35,50] The *APOE* ε4 allele, which encodes the APOE4 protein, is the second most common allele in the population (15%), after ε3 (77%) and ahead of ε2 (8%), but its frequency goes up to nearly 50% in AD patients, and its presence is associated with a 3–4 times higher risk of dementia and AD.[35] Studies with knock-out mice have shown that amyloidogenesis and Aβ deposition are dependent on the expression of the *APOE* gene, particularly the ε4 allele.[38] APOE4 contributes to AD pathogenesis by both modulating amyloid metabolism and interfering with lipid metabolism in the brain and thus synapse integrity.[35]

Besides cholesterol and other lipids, APOE also transports Aβ throughout the plasma and CNS[35] and promotes its endocytosis into CNS cells by interacting with membrane-surface receptors, mainly through the low-density lipoprotein receptor-related protein-1 (LRP1).[35,38,42a] The expression of the *APOE* ε4 allele is associated with increased $A\beta_{42}$ formation and aggregation, SNP formation, mitochondrial dysfunction, and tau-protein hyperphosphorylation, as well as decreased efficacy in both enzymatic and receptor-mediated Aβ clearance.[35] APOE4 is also the least effective isoform with respect to neuron and synapse maintenance and repair. Together, these features aggravate pathogenesis and neurotoxicity and thus have an unfavorable effect on the clinical status of AD patients. Indeed, patients with the *APOE* ε4 allele tend to show weaker recovery after brain damage in comparison with patients who express the *APOE* ε3 gene. The mutation of APOE ε4 has also been associated with increased risk of vascular dementia.[51] The poor ability of APOE4 to repair brain damage in combination with cumulative pathologic stimuli over an extended period of time form a solid foundation for the greater risk of LOAD in APOE4-positive individuals.

8. Pharmacological Basis for the Benefits of Statins in Alzheimer's Disease

Statin-mediated neuroprotection has received increasing attention during the last decade.[39,40,50b,52] Though controversial, this hypothesis provides new insight into CNS pathology. According to van der Most et al. (2009),[52e] statin-mediated neuroprotection is the sum of cellular and systemic mechanisms that oppose several pathological pathways of AD.

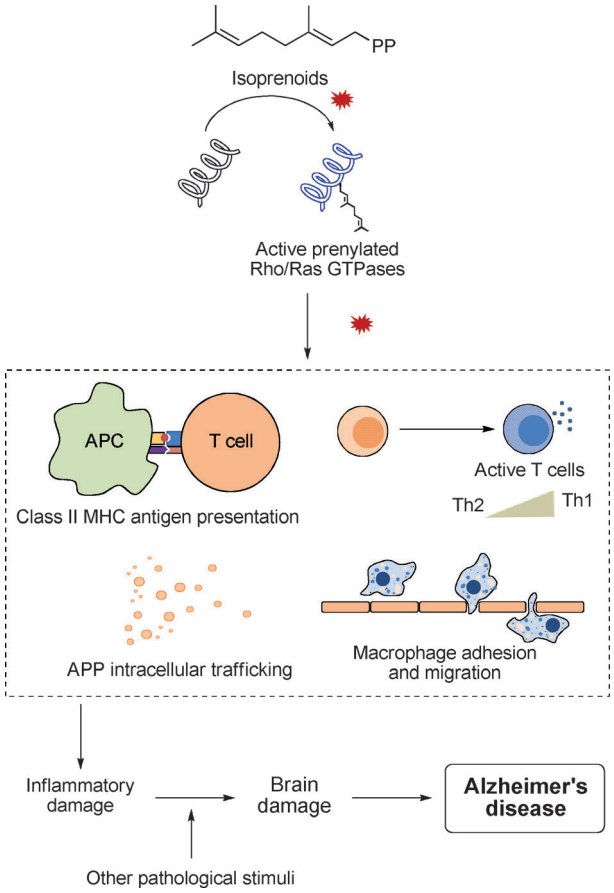

Figure 8. Role of isoprenoids in neurodegeneration and Alzheimer's disease. Isoprenoids activate immune cells and promote immune and inflammatory response through protein prenylation, which further contributes to brain damage. When associated with other pathological stimuli, such as excitotoxicity, tau hyperphosphorylation, and neurotransmitter depletion, these biochemical mediators cause brain damage and contribute to AD pathology in a significant way. APC = antigen-presenting cell.

8.1. Cellular Mechanisms for Statin-Associated Neuroprotection

8.1.1. Direct Effect on Brain Cholesterol

Statins in the prodrug lactone form are transported across the BBB through a simple diffusion mechanism, whereas those in the active acid form are transported by specific carriers in the BBB. Hence, both hydrophilic and lipophilic statins are able to directly lower brain cholesterol levels through local influence on brain tissue. However, lipophilic statins produce a greater reduction of brain cholesterol.[42b]

8.1.2. Lipid-Raft Destabilization

The lowering of cholesterol levels compromises the structural stability of lipid rafts and destabilizes the cholesterol-enriched membrane-associated microdomains in which amyloidogenesis occurs. A nonraft environment is favored as it leads to a decrease in the catalytic activity of β- and γ-secretase and promotes non-amyloidogenic APP cleavage by α-secretase. As a result, there is an overall decrease in the brain amyloid load.[52e,f]

8.1.3. Isoprenylation Blockage

The inhibition of HMG-CoA reductase also abolishes the synthesis of isoprenoid by-products and thus prevents protein isoprenylation, a post-translational modification essential to the activation of various cell types and cellular protein trafficking. Isoprenylation inhibition interferes with the intracellular trafficking of APP, so that less substrate is available to undergo amyloidogenic cleavage to Aβ. Immune-cell activation is also diminished, which prevents inflammatory damage.[52e,f]

8.1.4. Decrease in Leukocyte Adhesion Capacity

Another important feature is the statin-induced suppression of leukocyte and endothelium adhesion molecules, namely, LFA-1 (in leukocytes) and ICAM-1 (in the endothelium). The expression of these molecules is vital to the recruitment of inflammatory cells into the CNS. It has also been reported that statins can themselves bind to LFA-1, whereby they directly hinder the interaction of LFA-1 with endothelial ICAM-1 and further compromise the adhesion capacity of leukocytes. These immunomodulatory effects significantly reduce inflammatory brain damage.[52e,f,53]

8.1.5. Activation of Neuroprotective Pathways

Statins stimulate cellular neuroprotective signaling pathways, particularly PKβ/Akt, Wnt, and ERK, and increase the expression of brain-derived growth factor (BDGF). Furthermore, statins have demonstrated their antiapoptotic effect by blocking $A\beta_{42}$-induced neuronal death. Antiapoptotic signaling pathways are activated by treatment with statins, whereby the expression of caspase-3 is reduced. The pro-survival signaling pathways that are stimulated by exposure to statins are only beginning to be explored in AD models.[52e,f,53]

8.2. Systemic Mechanisms for Statin-Associated Neuroprotection

8.2.1. Plasmatic Cholesterol Depletion

Statins influence the amount of brain cholesterol indirectly by lowering cholesterol levels in the plasma. A decrease in plasmatic cholesterol creates a concentration gradient that promotes the efflux of brain cholesterol across the BBB as 24-S-OH-Chol. This "sink" effect enables the lowering of brain cholesterol levels in a passive and safe fashion.[42b]

8.2.2. Antioxidant Activity

The balance between the generation of reactive oxygen species (ROS) and antioxidant defense is believed to be disrupted in ageing and age-related diseases, such as AD, so that oxidative damage accumulates over the years.[5] Indeed, $A\beta_{42}$ inhibits complexes in the respiratory chain and lowers ATP levels. It thereby promotes mitochondrial dysfunction and further destabilizes antioxidant defense mechanisms.[5] Statins can effectively decrease oxidative damage by inhibiting several enzymatic systems associated with inflammatory

cells and ROS formation, namely, cyclooxigenase (COX), xanthine dehydrogenase and xanthine oxidase (XD/XO), neuronal and induced NO synthase, (nNOS and iNOS, respectively), and NADPH oxidase (NOX; NADPH is the reduced form of nicotinamide adenine dinucleotide phosphate, NADP).[52f]

8.2.3. Improvement of Cerebral Hemodynamics

Cerebral hypoperfusion is frequently observed in CNS pathology along with compromised cerebral hemodynamics. Statins can effectively improve vascular and endothelial function. First, statins increase the formation of nitric oxide (NO), a potent vasodilator, by promoting the activity of endothelial NO synthase (eNOS) while inhibiting the expression of ROS-generating forms of the enzyme (nNOS and iNOS).[52f,53] Second, statins induce the production of tissue plasminogen activator (tPA), which breaks the fibrin net essential to the coagulation process, and decrease the levels of procoagulant factors, such as plasminogen activator inhibitor (PAI-1), tissue factor, and thrombine.[52e] Studies have shown that statins improve cerebral hemodynamics and increase cerebral blood flow in animal models and human patients.[54]

8.2.4. Immunomodulation

Immune response is strongly connected with neurodegeneration and AD pathology. Statins exhibit significant immunomodulatory activity by interfering with the constitutive and induced expression of costimulators of major histocompatibility complex (MHC) class II molecules, T-cell proliferation and differentiation, and leukocyte recruitment into the CNS. T-cell differentiation into the noninflammatory Th2 phenotype is favored over proinflammatory Th1 cells.[52b,e,f]

9. Overview of Statin Clinical Efficacy against Alzheimer's Disease

We assessed the efficacy of statins as therapeutic agents against AD by searching biomedical databases (Pubmed, ISI Web of Science, The Cochrane Library, and the NIH Clinical Trials database) with "Alzheimer's disease", "statins", and "clinical trials" as keywords. Overall, the results obtained for this query are rather heterogeneous and require a thorough and detailed analysis. For the sake of accuracy, several factors need to be taken into account, namely, the type of statin used, the age of the individuals/groups enrolled, the stage of the disease, the duration of treatment, and the type of study performed. The ADCLT study,[55] a randomized double-blind study, identified a significant positive effect on the ADAS-cog performance in mild-to-moderate AD patients after 6 months of atorvastatin therapy (80 mg day^{-1}) as compared with the performance of the placebo group. These results are not in agreement with the findings of the LEADe trial,[56] in which atorvastatin in the same dosage was not associated with a clinical benefit over 72 weeks of treatment in patients who had been taking donepezil (10 mg day^{-1}) for more than 3 months prior to the screening. Another randomized double-blind placebo-controlled study conducted by Sano et al. (2011)[57] found that simvastatin had no positive effect on the results of mild-to-moderate AD patients. However, in this trial, subjects with normal lipid levels were enrolled. Open trials with AD patients found both lower levels of CSF biomarkers of the disease and slight increases in the ADAS-cog score of patients undergoing statin therapy with simvastatin, lovastatin, or pravastatin.[29] A similar outcome was observed with simvastatin (40 mg day^{-1}) or pravastatin (80 mg day^{-1}) in hypercholesterolemic subjects without dementia.[58] This last study suggests that statins may play a preventive role in the risk of disease development. This hypothesis is further supported by the findings of Carlsson et al. (2008),[59] who reported that simvastatin (40 mg day^{-1}) improved cognitive function in middle-aged adults whose parents suffered from AD. Together with the Rotterdam Study,[30] which found a 50% reduction in the risk of contracting AD upon treatment with statins, these results seem to indicate that statins play an active role in disease prevention rather than in the treatment of the disease when AD symptoms are already displayed. Thus, the modulation of cholesterol levels in middle age and/or statin administration prior to the appearance of dementia or cognitive impairment without dementia may have a better long-term outcome.[60]

10. Final Remarks and Future Perspectives

The association between cholesterol deregulation, statin therapy, and the risk of dementia and Alzheimer's disease remains controversial within neuroscience. Although epidemiological data clearly point towards an overall benefit of statin pharmacotherapy in AD patients, solid evidence from randomized clinical trials has not been gathered to date. The different ways in which clinical trials are structured and designed translate into heterogeneous results which fail to corroborate the conclusions of epidemiological studies. In particular, disparities in statin lipophilicity, treatment duration, CSF biomarkers, and statin dosage are the critical points behind the failure of HMG-CoA reductase inhibitors in these clinical trials. Nevertheless, the evidence gathered in this Review identifies statins as active pharmacological tools for disease prevention when AD symptoms are still absent. The control of midlife cholesterol levels is associated with a decreased risk of dementia and AD. As an additional beneficial stimulus, the pleiotropic effects of statins have a positive influence on various tissues and organ systems in the human organism. However, when neurological damage has passed the threshold to disease manifestation, statins are not a viable treatment option. Thus, the modulation of midlife cholesterol levels and/or statin administration prior to the appearance of dementia or cognitive impairment without dementia may have a better long-term outcome.

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