The Complex Actions of Statins in Brain and Their Relevance for Alzheimer's Disease Treatment: An Analytical Review

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Abstract: In view that several studies have shown a positive correlation between high cholesterol and an increase in the risk for developing Alzheimer's disease (AD) statins have been proposed as alternative drugs for its treatment and/or prevention. However, the potential benefits of statins remain controversial. Although they have lipid-lowering properties, statins also have pleiotropic effects that are unrelated to cholesterol reduction and have a wide range of biological implications whose consequences in brain function have not been fully characterized. In this work we analyze different studies that have reported both, beneficial and toxic effects for statins in the central nervous system (CNS), and we revise the literature that claims their potential for treating AD. First, we present an overview of the cholesterol metabolism and its regulation in the brain in order to provide the framework for understanding the pathological association between altered cholesterol and AD. Then, we describe the cholesterol-lowering and pleiotropic properties of statins that have been reported *in vivo* and in *in vitro* models. We conclude that the effects of statins in the brain are broad and complex and that their use for treating several diseases including AD should be carefully analyzed given their multiple and broad effects.

Keywords: Alzheimer's disease, amyloid beta, cholesterol pathway, HMG-CoA reductase, isoprenoids, neuroprotection, neurotoxicity, statins.

INTRODUCTION

Statins are competitive inhibitors of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase the rate-limiting enzyme in cholesterol biosynthesis [1, 2]. Discovered in 1976 by Endo and approved by the U.S. Food and Drug Administration (FDA) in 1987, statins are the most common prescribed drugs to lower systemic high cholesterol levels often associated with cardiovascular disorders [3]. Recently, statins have also been proposed for treatment and prevention of AD due to the association of this disease with altered cholesterol metabolism [4, 5]. However, epidemiological and experimental studies that have examined the beneficial effects of statins for AD prevention have yielded conflicting results [6, 7] In fact, recent studies have reported deleterious effects of several statins on neuronal function both, *in vivo* and *in vitro* [8-12].

The effects of statins on cholesterol metabolism in peripheral tissues are well known and include a variety of non-lipid lowering related or pleiotropic effects, which unveil the complexity of their molecular targets within the cell [13, 14]. However the impact of the chronic use of statins and the consequent side effects in the brain are not completely known.

To analyze the effects of statins in the CNS it should be kept in mind that cholesterol homeostasis in the brain is differentially regulated than in the periphery and that systemic cholesterol does not cross the brain blood barrier (BBB) [15]. In this work, we review the mechanisms that regulate cholesterol biosynthesis and metabolism in the brain and discuss the effects of statins when regulating these metabolic pathways. Our goal is to provide evidence on the mechanisms by which statins have been shown to modulate cholesterol in the brain and to analyze the grounds for some conflicting results.

CHOLESTEROL BIOSYNTHESIS AND ITS INHIBITION BY STATINS

Cholesterol is a structural component of the animal cell membrane, that modulates its physico-chemical properties [16]. It is also a key component of lipid rafts, membrane regions actively involved in signal transduction [17] and is the precursor of many pivotal molecules such as vitamin D, [18] and steroid hormones [19]. The cholesterol pathway also known as the mevalonate pathway involves the activity of more than 20 enzymes mainly located in the endoplasmic reticulum (ER) in a complex and highly expensivemetabolic route present in virtually all animal tissues [20]. The limiting step in cholesterol biosynthesis is catalyzed by the HMG-CoA reductase that transforms HMG-CoA to mevalonate which is then converted to an isoprenoid unit [21] (Fig. **1**). There is a growing number of intermediate compounds with biological activity in the cholesterol pathway, which unravels the extent of this metabolic route in regulating multiple cellular functions. In successive steps, isoprenoid units condense head to tail and form squalene, that is cyclized to yield cholesterol [22]. The bio-active intermediate compounds

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such as geranyl geranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP) are lipid tags used for post-translational modification of a wide variety of proteins, including the γ-subunit of heterotrimeric G proteins [23]. The prenylation of these proteins is required for their attachment to the cell membrane allowing the cell to exert biological functions such as migration, differentiation, proliferation and signaling [24]. The inhibition of the cholesterol pathway by statins also reduces the prenylation of G proteins such as Rho, Rac, Ras and Rab [25] which may have deleterious consequences for cell function [26]. In addition, isoprenoids are also involved in transcription (isopentenyl tRNAs), N-glycosylation (dolichol), and mitochondrial electron transport (ubiquinone and heme A) [22].
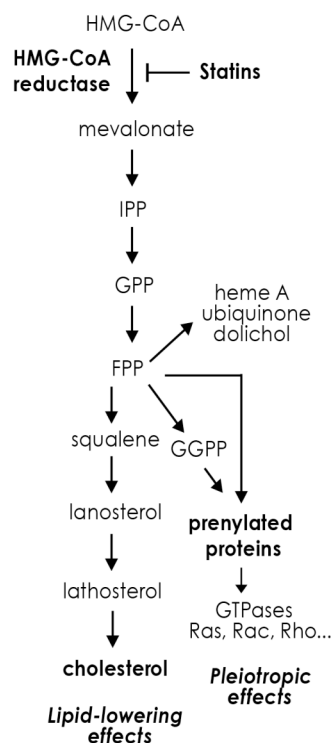

Fig. (1). Cholesterol biosynthesis pathway. HMG-CoA reductase inhibition by statins reduces cholesterol production and interferes with the formation of isoprenoids from mevalonate. Reduction in isoprenoids such as FPP and GGPP decreases prenylation of biological active proteins that may account for the broad and complex actions of statins.

REGULATION OF CHOLESTEROL SYNTHESIS

Systemic cholesterol homeostasis is controlled by synthesis, absorption of the dietary cholesterol intake, cellular efflux and degradation [27]. Biosynthesis of cholesterol is mainly regulated by its concentration in the ER [28] and is negatively modulated by plasma levels of low-density lipoprotein cholesterol (LDL-C) [29]. Much of the regulation of cholesterol synthesis takes place at this step [20] whereby the HMG-CoA reductase is subject to short and long-term regulation (Fig. **2A** and **B**). The short-term regulation of the enzyme occurs through its phosphorylation/dephosphorylation mediated by hormonal signaling. The phosphorylation of HMG-CoA reductase, which is triggered by glucagon through adenosine monophosphate-activated kinase (AMPK)

[30] decreases the enzyme's activity, while dephophorylation induced by insulin through protein phosphatase 2A (PP2A) increases its activity [31] (Fig. **2A**). Long-term regulation of HMG-CoA reductase is allosterically modulated by a negative feedback mediated by sterol contents (Fig. **2B**). A cholesterol sensing mechanism in the ER regulates the expression of genes involved in cholesterol biosynthesis (HMG-CoA reductase), as well as in its uptake (through LDL receptor) [28, 32] and in its degradation [33]. Sterol regulatory element binding proteins (SREBPs) are the classical transcription factors that regulate the homeostasis of cellular cholesterol controlling the expression of genes related with the synthesis of cholesterol and with its uptake from LDLs [28]. SREBPs are integral membrane proteins residing in the ER. In the presence of high sterol levels, SREBPs bind to another membrane protein, the SREBP cleavage-activating protein (SCAP) [34]. The complex SCAP-SREBP is retained in the ER through its binding to the insulin-induced gene-1 (INSIG-1)[35, 36]. When sterol levels decrease, the SCAP-SREBP complex leaves the ER and is transported to the Golgi via a secretory pathway [37]. In the Golgi, SREBP is sequentially processed by site-1 protease (S1P) and site-2 protease (S2P) [38, 39] that yield the soluble and active N-terminal domain of SREBP, which then enters the nucleus and induces the expression of their target genes [40]. This elegant regulation of cholesterol metabolism is consequence of a sterol-sensing domain (SSD) present in both, SCAP and HMG-CoA reductase [41]. At a post-translational level, sterols also regulate the degradation of HMG-CoA reductase [42-44]. When cholesterol is low, HMG-CoA reductase is stable, but when cholesterol increases, it interacts through its SSD domain with INSIG-1 [45] allowing its ubiquitination and its subsequent degradation by the endoplasmic-reticulum-associated protein degradation (ERAD) pathway [45].

ON THE COMPLEXITY OF THE EFFECTS OF STATINS

Statins are pharmacological agents used to lower systemic cholesterol levels. These drugs share an HMG-like moiety and competitively inhibit the HMG-CoA reductase, occupying a part of the substrate-binding site [1, 46]. There are six statins approved for clinical use by the FDA as cholesterol lowering agents [47] and are classified in two groups: lipophilic (lovastatin, simvastatin, fluvastatin) and hydrophilic (atorvastatin, pravastatin, rosuvastatin) being pravastatin the most hydrophilic and simvastatin the most lipophilic [48]. Lovastatin, simvastatin, pravastatin and mevastatin are natural compounds extracted from the fungi such as *Aspergillus terreus*, whereas fluvastatin, atorvastatin and rosuvastatin are synthetic molecules [48]. Lipophilic statins may cross the BBB but only lovastatin has been detected in the cerebrospinal fluid (CSF) at significant levels indicating that this statin may have potential CNS-related effects [49]. All statins except pravastatin are catabolized in the liver by the cytochromeP450 (CYP450) system [50, 51]. Although these drugs are generally well tolerated, they have been associated rarely with serious complications such as rhabdomyolysis, renal failure, depression, violence and paranoia in sensitive patients [52, 53]. The proposed mechanism associated with many of the adverse effects of statins appears
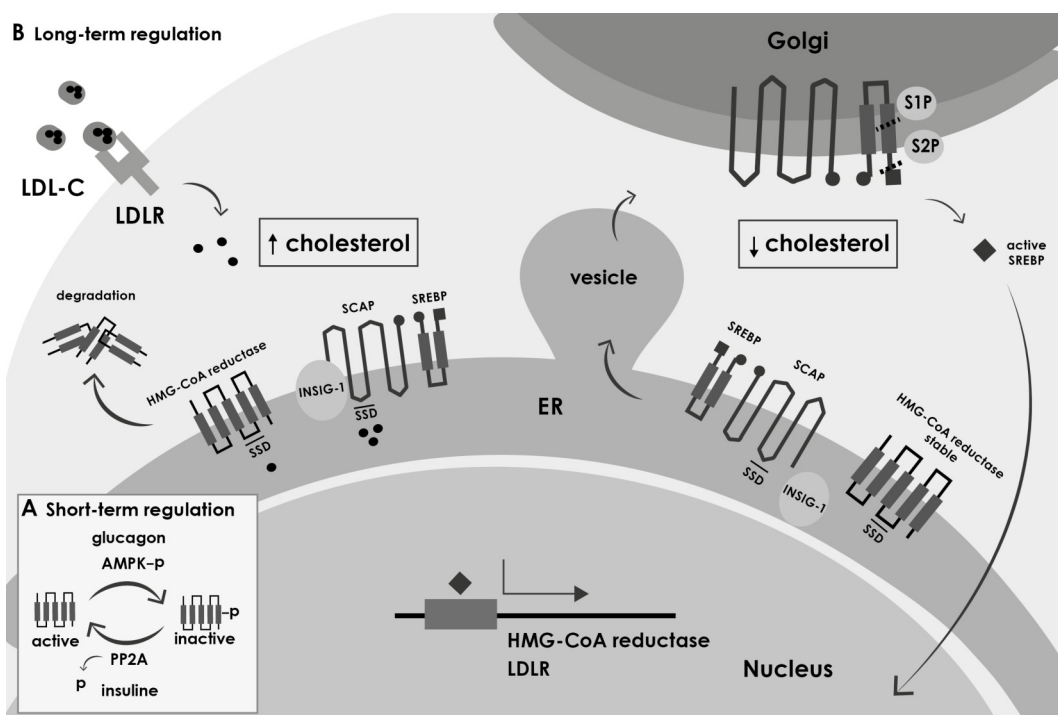
Fig. (2). Regulation of cholesterol synthesis. Biosynthesis of cholesterol is mainly regulated by its concentration in the ER. Cholesterol synthesis through HMG-CoA reductase is subject to short and long-term regulation. **A)** The white box at the bottom left corner shows the short-term regulation of cholesterol synthesis. The phosphorylation of HMG-CoA reductase, which is triggered by glucagon through AMPK decreases the activity of the enzyme, while dephophorylation induced by insulin through PP2A increases it. **B)** Long-term regulation of HMG-CoA reductase is allosterically modulated by a negative feedback mediated by cholesterol concentration. SREBPs residing in the ER are the transcription factors regulating the homeostasis of cellular cholesterol that control the expression of HMG-CoA reductase and LDLR. In the presence of high cholesterol (↑ cholesterol), SREBPs bind to SCAP. The complex SCAP-SREBPs is retained in the ER through its binding to INSIG-1. When cholesterol levels decrease (↓ cholesterol) the SCAP-SREBP complex leaves the ER and is transported to the Golgi via the secretory pathway. In the Golgi, SREBPs are sequentially processed by S1P and S2P yielding the soluble and active SREBPs, which then enter the nucleus inducing the expression of their target genes. Cholesterol also regulates the degradation of HMG-CoA reductase. When cholesterol levels are low HMG-CoA reductase is stable, but when cholesterol increases it interacts through its SSD domain with INSIG-1 allowing its degradation.

to depend mainly on the inhibition of the cholesterol synthesis pathway.

The multiple pleiotropic effects of statins not attributable to the reduction of LDL-C have received growing attention since they can explain some of the extra benefits derived from the their use [54]. Statins have anti-inflammatory, immunomodulatory, pro-angiogenic and anti-thrombotic effects [55, 56]. Therefore, the mechanism of action of statins in neurons is a fundamental issue in order to delineate their potential use for neurodegenerative disorders. A major comprehension of cholesterol metabolism in the brain is crucial to understand how it acts in nervous cells and if/how its pathological alterations can be prevented or repaired by statins.

BRAIN CHOLESTEROL METABOLISM

The brain is the organ with the highest contents of cholesterol. About 25% of the body cholesterol resides in the brain mainly in its unesterified form and is the major structural component of cell membranes and myelin sheaths [57]. In the CNS cholesterol also participates in key cellular process including cell differentiation, dendritic and synaptic formation, axonal guidance, neurotransmission, endocytosis,

synapse plasticity and intracellular signaling as a precursor of active neurosteroids [58, 59].

Significant differences in cholesterol metabolism between the CNS and the periphery have been described. The half-life of circulating cholesterol is only of few days in contrast to brain cholesterol that may remain for 6 months in rodent and 5 years in humans [57]. The metabolism of cholesterol in the CNS is rather poorly understood as compared to our knowledge of peripheral cholesterol.

In humans, maternal cholesterol is the primary source of brain cholesterol during early stages of development [60]. The formation and maturation of the BBB at 12–18 weeks [61] of gestation impedes the uptake of lipoproteins from circulation [62]. Thus, after this developmental stage, brain cholesterol levels are independent from dietary uptake or hepatic synthesis and its production essentially (>99%) depends on *denovo* synthesis [57].

During axonal myelination, oligodendrocytes have the most cholesterol synthesis capacity; later in adulthood, the rate of synthesis of these cells decreases and cholesterol continues being synthesized mainly by astrocytes [63, 64]. Neurons are able to synthesize most of the cholesterol needed for

their growth and synaptogenesis at early stages of development [59]. But in the mature brain, neurons reduce their biosynthetic capacity probably due to the high-energy expenditure of the pathway and become dependent of the cholesterol provided by astrocytes [65, 66].

Cholesterol homeostasis in the brain is maintained through the balance between cholesterol transport via a brain-specific lipoprotein-dependent process and the endogenously synthesized cholesterol. Blood is not a source of lipoproteins in the brain; these molecules are synthesized, assembled and secreted mainly by astrocytes and traffic back to neurons [67, 68]. The ATP-binding cassette (ABC) transporters are important molecules for mediating lipid transport in the CNS, especially in the formation of apolipoprotein E (APOE) [69]. Once cholesterol is synthesized, the ABC transporter A1 (ABCA1), which is expressed in both neurons and glial cells, exports it [70-72].

There is evidence showing that cholesterol biosynthesis in the brain is also regulated by SREBPs and HMG-CoA reductase in a similar way than in peripheral tissues through feedback inhibition of gene expression and protein degradation [73]. In neurons, HMG-CoA reductase is present and active in the ER but is also found in axons where cholesterol synthesis does not take place [74] suggesting that this enzyme could have other functions in the neuron. The balance of cholesterol levels in the brain is finely modulated. Cholesterol is excreted from the brain in the form of 24-hydroxycholesterol (24-OHC) by the action of the cholesterol 24-hydroxylase (CYP46) [57, 72, 75] a specific neuronal enzyme [76] and although circulating cholesterol does not cross the BBB, its oxidized product 27-hydroxycholesterol (27-OHC) can reach the brain [77, 78]. Based on the latter, it has been hypothesized that the influx of 27-OHC into the brain may represent a link between hypercholesterolemia and the risk to develop AD [79, 80].

CHOLESTEROL DYSREGULATION IN AD

Epidemiological and experimental studies have suggested that hypercholesterolemia increases the susceptibility to develop AD [81, 82] which is characterized by the accumulation in brain of amyloid beta (Aβ) protein and the hyperphosphorylation of the microtubule-associated protein tau. In fact, the presence of the ε4 allele of APOE, which is positively associated with high-cholesterol contents in brain, is one of best-established risk factors for developing AD [4, 83-85]. Interestingly, it has been found that high circulating cholesterol at middle-age increases the risk of AD by 66% [86] although at late-ages this correlation is no longer consistent.

Recently genome-wide association studies have identified polymorphisms in genes associated with cholesterol homeostasis, including ABCA1, ABC transporter A7 (ABCA7) and clusterin (CLU) [87-89]. Also high-plasma levels of 24-OHC have been associated with AD [90, 91] and vascular dementia [92]. However, it is still unclear whether high-plasma 24-OHC levels are related directly to the development of AD or if it is a reflection of the BBB disruption or both [93]. Most of the results in this respect have shown that cholesterol regulation in AD is lost at different levels: bio-

synthesis, transport, lipoprotein assembly, lipoprotein receptors, and signaling [94].

A body of evidence suggests that cholesterol can influence the activity of the enzymes involved in the metabolism of amyloid beta precursor protein (APP) and Aβ production. Studies in animals have shown that excess of dietary cholesterol accelerates Aβ accumulation in brain and in contrast, low-cholesterol favors the non-amyloidogenic pathway of APP metabolism decreasing Aβ production and amyloid plaque formation [95-98]. Although the mechanisms by which cholesterol affect Aβ production are not entirely clear, it has been suggested that changes in membrane cholesterol levels or distribution in lipid rafts influence the activity and expression of β- and γ-secretases involved in the amyloidogenic processing of APP [95, 97, 99-102]. However, epidemiological data could not explain the relationship between systemic high-cholesterol and Aβ accumulation. In fact, although in a cohort of non-demented elderly adults high circulating cholesterol levels were not directly related to Aβ deposition, genetic factors related with high cholesterol transport were associated with Aβ deposition in the brain [103]. On the other hand, high-cholesterol enhances neuronal toxicity of Aβ by increasing reactive oxygen species (ROS) formation [104, 105]. This way, cholesterol may increase the vulnerability of brain to toxic events in the elderly at two levels: promoting Aβ deposition and increasing its toxicity.

EFFECTS OF STATINS IN BRAIN CHOLESTEROL METABOLISM AND THEIR USE IN AD

Epidemiological studies have reported highly variable outcomes after statin administration in AD patients making it difficult to establish their potential beneficial use in the clinics [6, 7, 106-108]. It has been suggested that controversial results are probably due to multiple variations in the experimental design such as time and doses of statin administration, ability of statins to cross the BBB, age of subjects in the studies, basal levels of cholesterol, catabolism of statins in the liver and their interaction with xenobiotics that can alter their pharmacokinetics [109]. Because of the disruption of the BBB in AD it has been considered that both lipophilic and hydrophilic statins can access the brain, altering cholesterol metabolism differently among patients depending on the BBB status.

It is interesting to establish whether statins are able to affect cholesterol metabolism in the brain and to evaluate if, as a consequence, their potential therapeutic role may involve some pleiotropic effects. Studies in AD patients [110] and in animal models have shown that simvastatin or pravastatin reduces the levels of Aβ both in CSF and brain [111] and decreases *de novo* synthesisof cholesterol without altering the cerebral cholesterol contents [112]. In cultured neurons lovastatin also reduces Aβ but does not lower total cholesterol concentrations [113]. Lovastatin and simvastatin are able to reduce the levels of 24-OHC in plasma [114] and in CSF of AD patients [110]. In other reports, both statins have been shown to strongly reduce the levels of brain membrane cholesterol and affect its distribution in synaptosomal membranes [115, 116]. Experimental evidence has shown that lovastatin and the cholesterol extracting compound, methyl-β-cyclodextrin (MβCD) alter the function of protein complexes associated with lipid rafts in cultured neurons [99].

Furthermore, treatment with simvastatin induces the up-regulation of HMG-CoA reductase mRNA in the rodent brain [117]. Lovastatin and mevastatin reduce APOE secretion from glial cells through inhibition of protein prenylation [118] (Table **1**). Several statins (lovastatin, pravastatin, and simvastatin) also affect expression of genes involved in cell growth, signaling and cholesterol trafficking in the mouse brain [119]. Thus, many of the reported effects of statins in brain or cultured neurons depend on their ability to inhibit the cholesterol pathway. Despite the widespread use of statins, the consequences of inhibiting cholesterol synthesis in the CNS have not been elucidated. Some works using animal models and cell cultures have indicated the putative neuro-protection of statins [120]. However, other reports have shown some toxicity. Neuronal death induced by exposure to statins may depend on the reduction of cholesterol synthesis and on the decrease of other isoprenoids to critical levels such as to compromise neuronal function. The evidence pointing at the beneficial and deleterious effects of statins will be reviewed in detail below analyzing the role of statins as neuroprotectants *vs* their risk for inducing neurotoxicity.

NEUROPROTECTIVE POTENTIAL OF STATINS

As previously mentioned, different effects of statins on neuronal survival can be associated with their capacity for lowering cholesterol, but also with a variety of pleiotropic effects (Fig. **1**, Table **2**). Similarly, some groups have demonstrated that the pleiotropic functions of statins in AD likely play critical roles. For example, it has been proposed that the beneficial use of statins for treating this disease depends on a neurovascular protection [108] and on the reduction of oxidative and nitrosative stress [121].

a) Aβ Metabolism and Toxicity

It is now well established that changes in cholesterol levels result in modifications in Aβ production. The extraction of cholesterol from membranes using MβCD and lovastatin reduces Aβ production in hippocampal cultured neurons [99]. This may occur through posttranslational modifications since atorvastatin and simvastatin inhibit beta-site APP-cleaving enzyme 1 (BACE-1) dimerization [122] activity and protein levels [123] leading to a reduction of Aβ levels. Moreover, in AD patients clinically treated with simvastatin for 12 months, an increase in APP products related with the non-amyloidogenic process occurred [124]. In line with the previous mentioned study, lovastatin administration at low doses to human neuroblastoma cells, increases α-secretase activity favoring the non-amyloidogenic route [125]. Lovastatin also promotes the clearance of Aβ [126], modulates APP distribution in lipid rafts [127] and atorvastatin diminishes maturation and phosphorylation of APP [128]. In addition, lovastatin protects human neurons from Aβ toxicity by inhibiting glycogen synthase kinase 3 beta (GSK-3β) activity and by increasing nuclear translocation of transcription factors such as catenin (cadherin-associated protein), beta 1 (β-catenin), transcription factor 3 (TCF-3), and lymphoid enhancer binding factor 1 (LEF-1) required for neuroprotection [129]. Altogether these studies suggest that beyond the statins ability to inhibit cholesterol synthesis as the main mechanism for their anti-amyloidogenic action, other effects also may involve the activation of different metabolic signaling pathways.

b) Neuroinflammation

Activated astrocytes and microglia are frequently observed in AD suggesting a pivotal role of inflammation in

Table 1.   Statins' effects in brain cholesterol.

Effects	Model	Statin	Treatment	Ref.
Reduces levels of Aβ [109, 110, 112] and decreases de novo cholesterol synthesis (lathosterol) [111] without altering cholesterol contents. [111, 112]	AD patients' CSF	simvastatin	80 mg/day, 26 weeks	[109]
	CSF and brain of guinea pigs	simvastatin	0.5 % of diet, 3 weeks	[110]
	cultured hippocampal neurons	simvastatin or lovastatin	4 μM, 48 h or 72 h	
	cultured cortical neurons			
	guinea pig brain	simvastatin	150 mg/day, 3 weeks	[111]
		pravastatin	250 mg/ day, 3 weeks	
	SH-SY5Y (APPsw-293)	lovastatin	10 μM, 24 h	[112]
Reduces 24-OHC levels. [109, 111, 113]	AD patients' plasma	pravastatin	250 mg/ day, 3 weeks	[113]
Reduces levels of brain cells' membrane cholesterol.	mouse brain cells' membranes	lovastatin	100 mg/kg/day, 23 days	[114]
		simvastatin or lovastatin	80 mg/kg/day, 23 days	[115]
Up-regulates genes involved in cholesterol homeostasis (mRNA of HMG-CoA reductase and ABCA1).	mouse brain	simvastatin	100 mg/kg, 3 days	[116]
Reduces protein isoprenylation and APOE release.	BV-2 cells	lovastatin or mevastatin	2.5-10 μM, 16–24 h	[117]
	hippocampal slices	lovastatin	100 μM, 24-72 h	

Table 2.   Statins' neuroprotective effects in brain.

Effects	Model	Statin	Treatment	Ref.
a) Aβ metabolism and toxicity				
Decrease of cholesterol levels by MβCD and lovastatin induces a reduction in Aβ production and secretion	cultured hippocampal neurons	lovastatin	10 μM, 4 days	[98]
Inhibits the protein levels of BACE-1[122] its dimerization, [121] and activity. [121, 122, 126]	bHEK cells	lovastatin or simvastatin	1, 10 >10 μM,16 h	[121]
	aged dog brain	atorvastatin	80 mg/day, 14.5 months	[122]
Stimulates the non-amyloidogenic pathway of APP [123] by increasing the α-secretase [124, 194] activity.	CFS of AD patients	simvastatin	20 mg/day, 12 months	[123]
	SK-N-MC and SH-SY5Y cells	lovastatin	1 and 2 μM, 4 h	[124]
	NB2a cells (APPsw-695)	atorvastatin	5 μM, 24 h	[194]
Inhibits Aβ production through the reduction of APP in lipid rafts [126] and APP phosphorylation. [127]	cultured hippocampal neurons (lipid rafts fractions)	lovastatin	5-10 μM, 36 h	[126]
	cultured cortical neurons	atorvastatin or pitavastatin	0.2-2.5 μM, 4 days	[127]
Promotes degradation of Aβ via stimulation of IDE secretion.	BV-2 cells	lovastatin	5 μM, 24 h	[125]
Protects neurons from Aβ toxicity reducing GSK-3β activity and inducing Wnt signaling.	SK-NSH cells	lovastatin	4 μM, 24 and 48 h	[128]
b) Neuroinflammation				
Attenuates the production of inflammatory cytokines (MCP-1 [130] IL-1β, IL-6, and TNF-α[132]), reduces senile plaques, [130, 131] tau phosphorylation and improves cognitive function. [131, 132]	aged APP-Tg mouse	atorvastatin or pitavastatin	30 mg/kg/day, from 5–15 months of age (examined every 5 months)	[131]
				[130]
	rat hippocampus (impaired by intracerebroventricular injection of Aβ)	atorvastatin	5 mg/kg/day, 3 weeks	[132]
Improves cerebrovascular function and reduces glial activation as well as number of Aβ plaque-associated dystrophic neurites.	aged APP-Tg mouse	simvastatin	20 mg/kg/day, 8 weeks	[133]
Inhibits the actions of Rho GTPases through reduction in isoprenylation and attenuates Aβ-stimulated inflammation.	BV-2 cells	lovastatin or simvastatin	different doses,18 h	[134]
c) Antioxidant propierties				
Increases GSH levels[120] and glutamine synthetase activity [138]	aged dog brain	atorvastatin	80 mg/day, 14.5 months	[120]
	streptozotocin-induced model of AD in rats	simvastatin pravastatin	5 mg/kg, 4 weeks	[138]
Reduces lipoperoxidation, [120, 139] protein oxidation and nitration.[120] Prevents the memory impairment and oxidative stress (superoxide anion formation) that occurs after Aβ injection.[140]	aged APP-Tg mouse	atorvastatin or pitavastatin	unidentified	[139]
	mouse brain (impaired by intracerebroventricular injection of Aβ)	fluvastatin	5 mg/kg/day, 2 weeks	[140]

(Table 2) contd….

Effects	Model	Statin	Treatment	Ref.
d) Neuronal Survival and Plasticity				
Protects neurons from excitotoxic death (by NMDA[142] or glutamate[143]) through a reduction in the association of NMDAR1 to lipid rafts. [144]	cultured cortical neurons	rosuvastatin or simvastatin atorvastatin mevastatin pravastatin	100-300 nM, 6 days	[142]
	cultured cortical neurons	atorvastatin	1 μM, 2-4 days pre-treatment	[143]
	cultured cortical neurons	simvastatin	250 nmol/L, 4 days	[144]
Induces neuroprotection of glutamate-mediated excitotoxicity via activation of the TNF-R2-signaling pathway.	cultured cortical neurons	lovastatin	unidentified	[145]
Protects cells from PAF-induced neuronal damage by reducing PAF receptors.	cultured cortical or cerebellar neurons	simvastatin	100 nM, 24 h	[150]
Induces neurite outgrowth [151, 153] through activation of EGFR, up-regulation of NeuN,[151] and inhibition of RhoA signaling. [152, 153]	NB2a cells	mevastatin	5-10 μM, 24 h	[151]
	cultured hippocampal neurons	pravastatin	100 μM, 4-48 h	[153]
	PC12 cells	lovastatin	10 μM, 24 h	[152] F
Enhances neurite outgrowth by activation of Akt/mTOR pathway [154] and increases the number and length of dendritic branches by activation of GGTase-I and Rac1.[155]	cultured cortical neurons	atorvastatin	0.05-10 μmol/L, 48 h	[154]
	cultured cortical neurons	atorvastatin	0.1 μM, 30 min-24 h	[155]
Protects neurons after injury reducing neurological deficits; [156, 157] improves cerebral blood flow [156, 158] and induces angiogenesis, [157, 159, 160] neurogenesis [159, 160] and synaptogenesis. [159, 160]	Traumatic brain injury (TBI), rat brain	atorvastatin or simvastatin	320 mg/kg, 14 days	[156]
	TBI, rat brain	atorvastatin or simvastatin	unidentified, 14 days	[157]
	stroke-prone spontaneously hypertensive rats	atorvastatin	2 mg/kg and 20 mg/kg, 11 weeks	[158]
	middle cerebral artery occlusion (MCAo), rat brain	atorvastatin	1 and 3 mg/kg,14 days	[159]
			10 mg/ kg, 14 days	[160]

neuronal death [130]. Thus, it has been proposed that the antinflammatory actions of statins might contribute to neuroprotection. According to the above, animals from a transgenic mouse model of AD that chronically received atorvastatin or pitavastatin, showed a reduced number of activated microglia as well as a decrease in the mean of the area occupied by senile plaques, a diminishment in phosphorylated tau [108, 131] and an improvement in cognitive performance [132]. Similarly, atorvastatin diminishes the memory impairment produced by intracerebroventricular injection of Aβ in rats, which correlates with a reduction in the levels of the inflammatory cytokines interleukin 1beta (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) in brain [133]. Simvastatin promotes cerebral blood flow and reduces oxidative stress, glial activation and Aβ plaques associated to dystrophic neurites [134]. Some of these effects have been ascribed to different mechanisms not necessarily related to

the statin-dependent cholesterol lowering effect. For example, simvastatin attenuates Aβ-induced microglia activation and IL-1β induction through the diminishment in isoprenylation of Rho family members [135]. On the other hand, it has also been found that in cultured hippocampal slices, statins provoke microglial activation and upregulation of TNF-α dependent on the inhibition of the mevalonate pathway and on the generation of GGPP [136].The question of whether activated microglia positively or negatively contributes to AD progression is also unresolved. Thus, the anti-inflammatory actions of statins may be mostly indirect and the underlying molecular mechanism of action has not been elucidated yet.

c) Antioxidant Properties

The presence of oxidized proteins, lipids and DNA has been identified in tissues from AD patients, indicating an

undergoing oxidative stress process [137, 138]. The antioxidant properties of statins represent one of their pleiotropic effects. A recent study showed that atorvastatin administration significantly reduces lipoperoxidation, protein oxidation and nitration and that it increases glutathione (GSH) levels in the parietal cortex of aged beagles [121]. Similarly, in a streptozotocin model of AD, simvastatin and pravastatin prevented the decrease in GSH contents and in glutamine synthase activity in the rat hippocampus [139]. Atorvastatin and pitavastatin reduce the levels of lipid oxidation the 4-hydroxynonenal (4-HNE) product and of advanced glycation end products (AGEs) in AD mice brains [140]; and fluvastatin prevents memory impairment and oxidative stress that occurs in mice after Aβ injection [141]. Therefore, the reduction of oxidative damage in AD may be important for achieving the beneficial effects attributed to statins in regard to cognitive function.

d) Neuronal Survival and Plasticity

Excitotoxicity is characterized by excessive calcium entry into the neurons mediated mainly by overactivation of the N-methyl-D-aspartate (NMDA) glutamate receptor subtype and might contribute to neuronal death in some neurodegenerative conditions [142]. Several statins are able to protect cultured neurons from NMDA excitotoxicity through different mechanisms. Zacco *et al.,*[143] showed that in cortical cultured neurons, different statins protect cells from NMDA toxicity seemingly by cholesterol depletion. In another work, Bosel *et al.,*[144] found that atorvastatin attenuated glutamate-induced excitotoxicy through the decrease of the NMDA receptor function and independently from the inhibition of the HMG-CoA reductase. Statins, in particular simvastatin have been also shown to reduce the association of the subunit 1 of NMDA receptors (NMDAR1) to lipid rafts [145], thus providing evidence for different mechanisms of neuronal protection. In addition, it has been reported that neuroprotection against excitotoxicity by lovastatin depends on the activation of the tumor necrosis factor receptor 2 (TNF-R2) signaling pathway [146].

High levels of the platelet-activating factor (PAF) have been found in AD brains [147]. PAF is a potent phospholipid mediator that is released under several pathological conditions [148] and mimics the toxicity induced by Aβ [149, 150]. Simvastatin is able to protect against PAF-induced neuronal damage reducing the number of PAF receptors in the lipid rafts, thus desensitizing neurons to PAF signaling [151].

Statins promote neurite outgrowth and neuronal plasticity through the activation of several signaling pathways, which are independent of cholesterol. For example, mevastatin induces neurite outgrowth by activating the epidermal growth factor receptor (EGFR) [152], while lovastatin and pravastatin enhance neurite outgrowth and cell differentiation by inhibiting the prenylation and consequently the activity of the small GTPase protein, RhoA [153, 154]. Atorvastatin also induces neurite outgrowth by activating the Akt/mTOR pathway in cortical neurons [155] and increases the number and length of dendritic branches by activating geranylgeranyltransferase type I (GGTase-I) and Rac1 in cortical cultured neurons [156]. Simvastatin and atorvastatin have also shown

to protect neurons after a traumatic brain injury in rats [157, 158] and to improve cerebral blood flow in stroke-prone spontaneously hypertensive rats [159]. All these positive effects were dependent on the activation of molecules involved in angiogenesis, neurogenesis and synaptogenesis [160, 161].

NEUROTOXIC EFFECTS OF STATINS

Although a number of studies have described some general neuroprotective effects of statins *in vivo* and *in vitro*, clinical evidence for their efficacy in AD is still not well accepted and their prescription should be carefully considered. The therapeutic use of statins have yielded conflicting results and there is general agreement that beyond the beneficial effects mentioned above, some toxic events, which are listed in (Fig. **1**) and (Table **3**) are also derived from their administration.

a) Negative Implications in Neuronal Survival and Plasticity

Growing evidence confirms the neurotoxicity of some statins under certain administration schemes, although at present the main mechanism leading to toxicity has not been established. Lovastatin is neurotoxic for developing human CNS cells at pharmacological concentrations [162]. However, it remains unclear whether this neurotoxicity depends on a decrease in cholesterol contents or if it is due to a reduction of other isoprenoid products. It has been suggested that the neurotoxicity induced by mevastatin depends on the reduction of internal cholesterol levels since neurotoxicity could not be prevented after adding non-esterol metabolites [163]. It is worth mentioning that lovastatin, being a statin prone to cross the BBB is also neurotoxic to human neuroblastoma cells and is associated with an increase in ROS production [105].

Multiple mechanisms have been linked to statins' neurotoxic effects. In differentiated neuroblastoma cells, exposure to mevastatin inhibits proteasome activity and induces degeneration [164]. In the human neuroblastoma line SH-SY5Y, lovastatin induces apoptosis in a time and dose-dependent manner associated with the reduction of mevalonate [165]. In the same cellular line it was demonstrated that lovastatin induced apoptosis through the mitochondrial pathway via the activation of caspase-9 as initiator and caspase-3 as effector [166]. Thus, although in many instances the beneficial effects of statins have been correlated with reduced levels of isoprenoids [13] there is also evidence that such reduction may lead to neurotoxicity. GGPP and FPP are crucial for post-translational modifications of small GTPases [167]. In the CNS, Rho GTPases participate in cytoskeleton remodeling and are implicated in neuronal development, migration, plasticity and protection [168-170]. Lovastatin and simvastatin reduce FPP and GGPP levels in both, cultured neurons [171] and in mouse brain [172] decreasing signal transduction pathways crucial for neuronal growth and survival. Lovastatin, mevastatin and atorvastatin treatment at high concentrations have been shown to inhibit neurite growth and proliferation and to reduce the viability of differentiated neuroblastoma NB2a cells [173]. In fact, HMG-CoA reductase inhibition by atorvastatin has also been

Table 3.   Statins' neurotoxic effects in brain.

Effects	Model	Statin	Treatment		Ref.
a) Negative implications in neuronal survival and plasticity					
Induces neuronal death [161, 162, 165] by apoptosis through the mitochondrial pathway via caspase activation [165] and through the reduction of Bcl-2 and Bcl-xL protein levels.[195]	human embryonic brain cells	lovastatin	0.01-1000 ng/ml, 3 h-12 days		[161]
	cultured cortical neurons	mevastatin	5 µM, 48 h		[162]
	SH-SY5Y cells	lovastatin	10 µM, 24 h		[165]
	rat brain neuroblasts	lovastatin	1-10 µM, 24 h or 48 h		[195]
Inhibits neurite outgrowth [172, 173] and cell death [172, 174] by mechanisms that involve reduction in GGPP, [173, 174] as well as reduction in the expression and function of RhoA. [174]	PC12 cells	atorvastatin	7 µM, 24-72 h		[173]
	cultured cortical neurones		8 µM, 48 h		
	NB2a cells	lovastatin or mevastatin atorvastatin	1, 3, 10, 100 µM, 24 h		[172]
	cultured cortical neurons	pravastatin	300 µM, 72 h		[174]
Induces alterations in the microtubule system [175, 176, 196] by increasing tau phosphorylation and inducing axonal degeneration [176] linked to the lack of GGPP[175] or cholesterol.[176, 196]	cultured hippocampal neurons	lovastatin	10 µM, different times		[175]
	cultured cortical neurons	mevastatin	300 nM,	65 h	[176]
				24, 48 or 72 h	[196]
Other mechanisms of neurotoxicity: increase of ROS production,[104] inhibition of the proteasome activity,[163] microglia activation and TNF-α up-regulation.[135]	human neuroblastoma cells	lovastatin	50-100 µM, 24 h		[104]
	N2a cells	mevastatin	40-150 µM, 24 h		[163]
	rat cultured hippocampal slices	mevastatin	10 µM, 6 days		[135]
b) Adverse psychiatric effects					
Produces cognitive deficts [178] and adverse psychiatric effects (somatization, depression, [52] and anxiety [11]).	patient survey-based analysis (34-86 years)	various	various		[178]
	hypercholesterolemic patients	simvastatin	20 mg/day, 12 weeks		[52]
	guinea pigs	atorvastatin or	0.5mg/kg, 6 weeks		[11]
		simvastatin	1 mg/kg, 6 weeks		
c) Neurotransmission Impairment					
Lowers number of synapsis [9] and impairs synaptic vesicle release.[9, 182]	cultured hippocampal neurons	lovastatin	0.25 µM, 7 days		[9]
	cultured hippocampal neurons	mevastatin	4 µM, 7 days		[182]
Interferes with synaptic plasticity reducing the expression of synaptic proteins, NMDAR currents,[8] long-term potentiation [185] and evoked post-synaptic currents. [183] Alters the function and dynamics of the serotonin system.[10]	cultured cortical neurons	mevastatin	300 nM, 21 days		[8]
	hippocampal slices	mevastatin	25 µM, 60 min		[185]
	cultured hippocampal neurons	mevastatin	4 µM, 6 h		[183]
	CHO cells (human 5-HT1A)	mevastatin	50 µM, 56 h		[10]

shown to cause neurite loss by interfering with GGPP synthesis in cultured neurons [174]. In cortical cell cultures, pravastatin-induced neurotoxicity was prevented by its co-treatment with GGPP, which restores the amount of the active RhoA protein. [175]. Lovastatin also produces degenerative changes affecting the neuritic network and altering the microtubule system, again due to the lack of the GGPP

[176]. In line with the previous finding, it has been shown that mevastatin alters microtubule stability by increasing tau phosphorylation and inducing axonal degeneration in cultured neurons [177]. All these effects were associated to a cholesterol deficiency since they could be reverted by incubating the cells with VLDL or cholesterol. At present the only explanation for the discrepancy between neuroprotec-
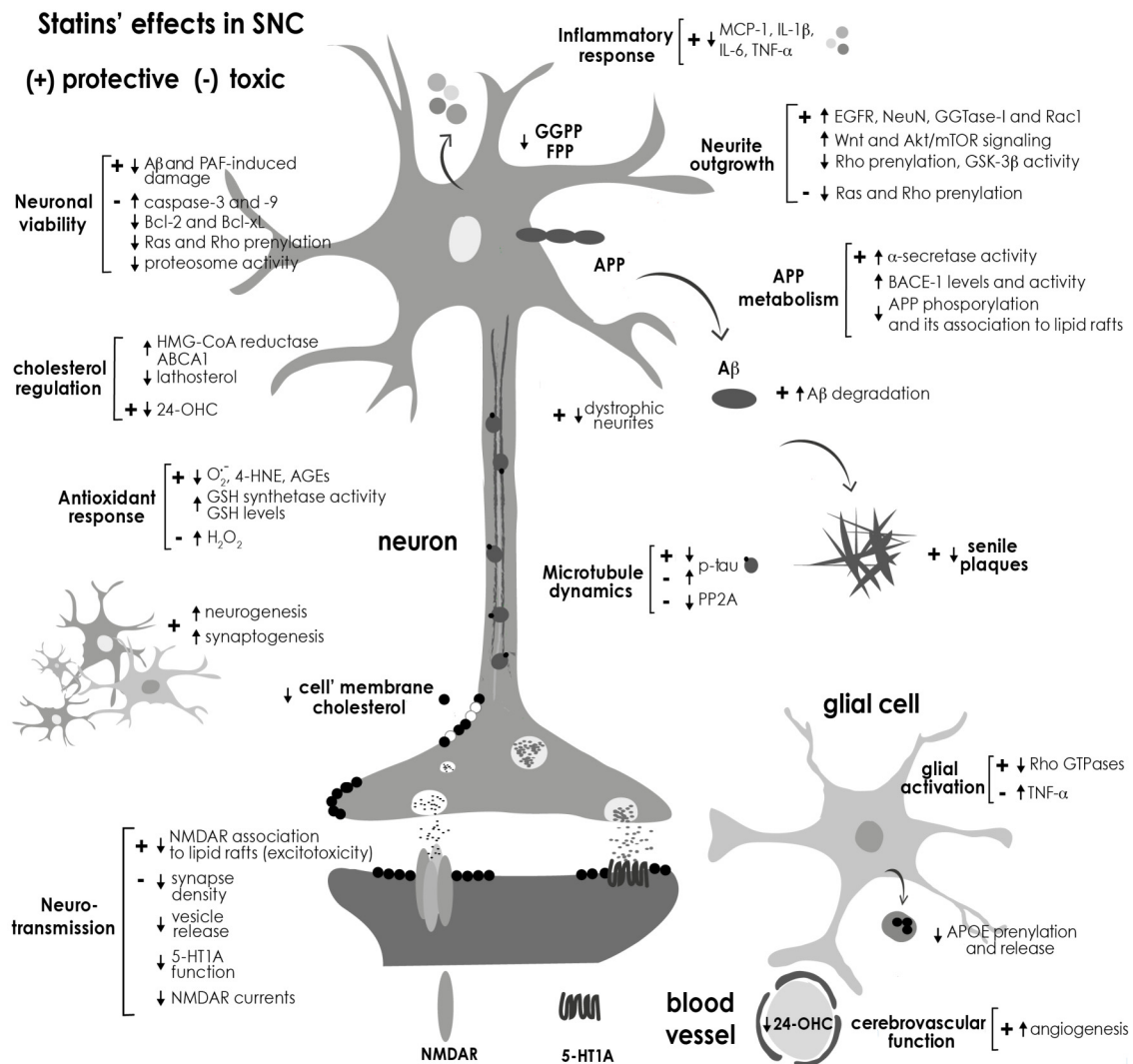
Fig. (3). Complex and broad effects of statins in CNS. The implications of statins' effects in the CNS are not completely understood. Protective or beneficial effects as well as detrimental effects have been reported after statins' administration. The plus sign (+) indicates a protective or beneficial effect in different cellular processes. The minus sign (-) represents the toxic effects of statins. Orientation of arrows indicate if statins upregulate (↑) or downregulate (↓) the depicted molecule levels as well as a cellular particular processes.

tion and neurotoxicity elicited by statins may involve differences in the doses and incubation times that are analyzed in the (Tables **2** and **3**). Although the majority of studies are experimental and involve *in vitro* or *in vivo* animal models, the problem about statins having a positive or a negative contribution in AD progression remains unresolved.

b) Adverse Psychiatric Effects

Given the complexity in the behavioral effects provoked by the use of statins, the FDA has approved to change the security labels for statins prescription indicating that they may cause memory loss and confusion during medication that can however be reversible once the drug is no longer administered [178-180]. Other reported effects of statins in patients are risk of depression, aggressiveness [12, 181] and somatization [52]. In an animal model, a low dose treatment with simvastatin or atorvastatin induced mild but significant levels of anxiety-related behaviors [11]. Although at present the mechanisms by which a long-term statin treatment leads

to cognitive and emotional alterations are not well understood, recent evidence suggests some molecular mechanisms that can be associated with neuronal dysfunction.

c) Neurotransmission Impairment

In neuronal membranes, cholesterol modulates synaptic function and neurotransmitter release. Depletion of cholesterol with MβCD [182] or with mevastatin in cultured hippocampal neurons impairs synaptic vesicle release [183]. Lovastatin lowers the number of synapses and also reduces synaptic vesicle release [9], while mevastatin affects cortical neuronal morphology and synaptic protein expression, reduces NMDAR currents [8] and decreases evoked postsynaptic currents [184]. Cholesterol imbalance induced by MβCD [185] or mevastatin reduces long-term potentiation in rat hippocampal slices [186] and chronic cholesterol depletion by mevastatin results in a significant reduction and functionality of the human serotonin H-1A receptors (5-HT1A) expressed in CHO cells [10]. Such depletion could explain,

at least in part, some of the psychiatric effects related to the chronic treatment with statins. Moreover, it has been suggested that lowering cholesterol beyond certain levels may inhibit the release of neurotransmitters at synapses and disrupt neuronal function [187, 188]. In a recent study Schilling *et al.,* [189] reported that the membrane lipid raft associated proteins syntaxin-1α and synaptophysin are altered after atorvastatin treatment in rats. Another report has also indicated that disruption of lipid rafts by mevastatin and fumonisin B1 (an inhibitor of the sphingolipid synthesis) induce depletion of excitatory and inhibitory synapses, loss of dendritic spines, and instability of membrane alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in cultured neurons [190]. These events may account for the adverse effects of statins on cognition.

d) Age-related Vulnerability of Statins Consumption

The relation between statins and improvement of cognitive function in the elderly is currently a matter of debate. The use of statins has been questioned given the reported adverse effects in this population [178]. An increase in undesirable side effects may include the age and genetic profile of the patient, as well as the presence of some associated diseases that may affect drug action and pharmacokinetics [191]. During aging, hippocampal cholesterol synthesis decreases but total cholesterol brain contents remain stable [192]. Chronic treatment with atorvastatin reduces the hippocampal volume in AD patients [193] who may be particularly susceptible to the adverse effects of statins due to the altered cholesterol metabolism and signal transduction dysregulation present in the disease [194]. However, it should be mentioned that some clinical studies do not support the idea that cognitive considerations should be a factor to discontinue statin medication for cardiovascular and cerebrovascular disease [195].

CONCLUSION

From the predicted increase in cardiovascular diseases in the coming years, it is expected that the prescription and use of statins will aggressively rise. Thus, the need to regulate statins' consumption, in order to avoid their abuse and eliminate self-medication represents a priority. In regard to medical practice it is highly relevant to carefully consider the clinical condition of the patients, as well as their age and genetic background before selecting a particular statin for its administration. Commonly, statins are prescribed for a long time, often for a life-time. Therefore it is worth considering that statins act locally by lowering lipid levels, but may have broad pleiotropic effects, thus affecting peripheral tissues and the CNS.

Currently, there is no solid evidence to support the use of statins for the treatment or prevention of neurodegenerative diseases such as AD; even when experimental and clinical studies have suggested positive effects on their administration for treating the disease, negativeoutcomes may rely upon the side effects mediated by a general inhibition of the mevalonate pathway. Data pointing at the effectiveness of statins for the treatment of AD are somewhat contradictory and further research is required to clarify their beneficial

and/or toxic effects and to determine if they can be safety used to prevent or treat AD.

ABBREVIATIONS

ABC transporters	=	ATP-binding cassette transporters
ABCA1	=	ABC transporter A1
ABCA7	=	ABC transporter A7
AD	=	Alzheimer's disease
AGEs	=	Advanced glycation end products
AMPA	=	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPK	=	Adenosine monophosphate-activated kinase
APOE	=	Apolipoprotein E
APP-Tg mouse	=	APP transgenic mouse
APP	=	Amyloid beta precursor protein
APPsw	=	APP Swedish mutant
Aβ	=	Amyloid beta
BACE-1	=	Beta-site APP-cleaving enzyme 1
BBB	=	Brain blood barrier
CLU	=	Clusterin
CNS	=	Central nervous system
CSF	=	Cerebrospinal fluid
CYP450	=	Cytochrome P450
CYP46	=	Cholesterol 24-hydroxylase
EGFR	=	Epidermal growth factor receptor
ER	=	Endoplasmic reticulum
ERAD	=	Endoplasmic-reticulum-associated protein degradation
FDA	=	U.S. Food and Drug Administration
FPP	=	Farnesyl pyrophosphate
GGPP	=	Geranyl geranyl pyrophosphate
GGTase-I	=	Geranylgeranyltransferase type I
GSH	=	Glutathione
GSK-3β	=	Glycogen synthase kinase 3 beta
HMG-CoA	=	3-hydroxy-3-methylglutaryl-CoA
IDE	=	Insulin-degrading enzyme
IL-1β	=	Interleukin 1beta
IL-6	=	Interleukin 6
INSIG-1	=	Insulin induced gene-1
IPP	=	Isopentyl pyrophosphate
LDL-C	=	Low-density lipoprotein cholesterol

LEF-1	=	Lymphoid enhancer binding factor 1
MCP-1	=	Monocyte chemotactic protein 1
MβCD	=	Methyl-β-cyclodextrin
NeuN	=	Neuronal nuclei
NMDA	=	N-methyl-D-aspartate
NMDAR1	=	Subunit 1 of NMDA receptor
PAF	=	Platelet-activating factor
PP2A	=	Protein phosphatase 2A
ROS	=	Reactive oxygen species
S1P	=	Site-1 protease
S2P	=	Site-2 protease
SCAP	=	SREBP cleavage–activating protein
SREBP	=	Sterol regulatory element–binding protein
SSD	=	Sterol-sensing domain
TCF-3	=	Transcription factor 3
TNF-R2	=	Tumor necrosis factor receptor 2TNF-α, tumour necrosis factor alpha
VLDL	=	Very-low-density lipoprotein
β-catenin	=	Catenin (cadherin-associated protein), beta 1
24-OHC	=	24-hydroxycholesterol
27-OHC	=	27-hydroxycholesterol
4-HNE	=	4-hydroxynonenal
5-HT1A	=	Serotonin H-1A receptors

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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