Neuroprotective Role of Statins in Alzheimer's Disease: Anti-Apoptotic Signaling

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Abstract: Alzheimer's disease (AD), a severe form of senile dementia is a neurodegenerative disorder. One of the most well characterized hallmarks of AD are extra-neuronal aggregates of amyloid-beta peptide (Aβ), known as amyloid plaques. Recent epidemiological studies suggest a link between statin intake, and a lowered incidence of AD. Statins are 3-hydroxy-3-methylglutaryl co-enzyme reductase (HMG) inhibitors, which are one of the most commonly prescribed drug groups used to lower serum cholesterol levels in patients with heart disease. Some of the pleiotropic effects of statins which are gaining attention are its ability to reduce Aβ production and deposition, inhibit caspase-3 mediated apoptosis, and demonstrate anti-inflammatory properties by reducing interleukin-6 (IL-6) levels. The molecular mechanisms responsible for the pleiotropic effects of statins in promoting neuronal survival are not fully understood. Our own research has shown that statins promote anti-apoptotic responses against Aβ-neurotoxicity through β-catenin-TCF/LEF signaling however, other anti-apoptotic statin mediated signaling pathways may also be involved. This review will describe AD pathogenesis, Aβ production, and the role of statins in mitigating these effects.

Keywords: Statins, Alzheimer's disease, anti-apoptotic signaling, neurotoxicity.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that affects nearly 30 million individuals worldwide [1]. AD patients experience progressive decline in their cognitive functions and loss of short-term memory with age. The brain regions most commonly affected in AD are the hippocampus and entorhinal cortex, both of which are involved in short-term memory and learning processes [2]. Histopathological examination of AD brain slices reveal amyloid-beta peptide (Aβ) deposits and neurofibrillary tangles (NFT), both hallmarks of AD. Amyloid deposits or amyloid plaques are mainly extra-cellular aggregates of amyloid beta peptides. NFTs, which are intra-cellular aggregates of the microtubule-associated protein Tau, also contribute to AD pathology. Both these deposits contribute to the neuronal loss observed in AD. Approximately 90% of AD cases are considered to be sporadic or not genetically linked. Sporadic AD also known as late-onset is often diagnosed after 65 years of age [3]. The remaining 10% of AD cases have a genetic component and are autosomal dominant in transmission [4,5]. In the familial form of AD (FAD), mutations in the APP gene promote enhanced Aβ production. Apart from APP, other factors such as mutations in presenilins (PS1 and PS2), and the presence of the APOE allele, APOEε4 (APOE is a apolipoprotein which transports cholesterol from the brain) promotes increased Aβ plaque load [4]. Interestingly, some patients with sporadic AD also display polymorphisms for the APOE gene therefore APOE is considered a risk factor rather than a genetic determinant of AD [4, 6]. Alteration in amyloid precursor protein (APP) processing leads to the accumulation of Aβ though a number of proteolytic cleavage processing products of APP. FAD is considered early-onset AD, with individuals displaying cognitive impairments often by the age of 65 [4].

Aβ peptide is a neurotoxic agent, which at high concentrations promotes neuronal loss [7]. The neurotoxic effect of Aβ has been demonstrated *in vitro*, in animals and in humans [8,9]. Aβ promotes neuronal loss by apoptosis [10]. A number of biological mechanisms have been implicated in neuronal loss these include: inflammation, free radial formation (reactive oxygen species), and glutamate mediated excitotoxicity [11].

Epidemiological research has shown a correlation between increased cholesterol levels (hypercholesterolemia) and the incidence of AD [12,13]. Hypercholesterolemia is increasingly being accepted to have a modulatory role in AD progression. Interestingly, Down Syndrome (DS) affected individuals having cholesterol levels of ≥200mg/dL carry a greater risk of developing AD [14]. This suggests that duplication of APP and higher than normal levels of cholesterol contribute to the development of AD. High cholesterol diets

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1874-0820/10

have been shown to increase Aβ deposition in mice [15,16]. In the brain, cholesterol is synthesized *de novo* through a multi-enzyme cascade that includes β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) synthase and HMG CoA reductase. Lowering cholesterol levels in patients using HMG-CoA reductase inhibitors, statins, has been associated with decreased Aβ levels in cerebral spinal fluid of patients with mild AD [17]. HMG-CoA reductase is the rate-limiting enzyme of cholesterol biosynthesis. In the liver, inhibition of HMG-CoA reductase stimulates low density lipoprotein (LDL) receptors, resulting in an increased clearance of LDL from the bloodstream and reduced blood cholesterol.

Ever since the association between statin use and lowered incidence of AD was demonstrated, research has focused on confirming these observations and elucidating the mechanism(s) by which statins might work to reduce AD symptoms. Statins have been used for lowering cholesterol levels and reducing the risks of both heart attack and stroke; however, there are numerous pleiotropic effects of statins which have yet to be explained. Understanding how statins lower Aβ levels and how they activate pro-survival signaling are two key areas of AD research. Our review describes the current literature surrounding the use of statins in mitigating AD pathology and suggests possible anti-apoptotic pathways that may be involved in neuroprotection.

STATINS

Statins aka HMG-CoA reductase inhibitors are a class of drugs that are used to reduce cholesterol biosynthesis. There are numerous statins in use today which are prescribed primarily for heart disease and stroke. Historically they were derived from fungus, but more recent versions have been synthesized chemically to increase potency and efficacy. Statins work by inhibiting the rate limiting step in cholesterol biosynthesis; they block the action of the enzyme HMG-CoA reductase. The reduced cholesterol synthesis leads to an up-regulation of LDL receptors in the liver, resulting in increased clearance of LDL from the bloodstream. Statins may be classified into three categories based on their increasing potency and efficacy in reducing low density lipoprotein cholesterol (LDL-C). First generation statins include lovastatin, pravastatin, and fluvastatin. Second generation statins include, simvistatin and atorvastatin. Third generation statins include, rosuvastatin [18]. A number of studies have suggested that brain cholesterol levels are not affected by statin intake since most of the cholesterol synthesis in the brain is *de novo*. However, it has been shown that high doses of simvastatin are able to decrease the cholesterol turnover in the brain resulting in decreased plasma concentrations of 24S-hydroxycholesterol [19].

APP PROCESSING

APP is a type-I single membrane spanning protein with a large extra-cellular amino terminal domain (NH2), and a short intra-cellular carboxyl domain (COOH) (Fig. **1a**). The APP gene lies on chromosome on chromosome 21q21.2-3. Genetic anomalies of the APP gene have been associated with a loss of cognitive function in transgenic mice [20]. The mode of inheritance of AD is autosomal dominant [4]. In FAD, mutations in the APP gene at the secretase cleavage sites are responsible for increased production of toxic Aβ fragments.

APP has numerous cellular functions, these include: functioning as a G-protein-coupled receptor, playing a role in cell adhesion, aiding synaptic transmission, and involvement in synaptic plasticity [21]. These have been demonstrated using APP knockout (KO) mice. One study showed that apart from having deficits in grip strength and locomotor activity learning, APP KO mice also showed age-dependent deficits in memory [22]. Alternative splicing of the pre-mRNA of APP results in the production of three isoforms: APP_{695}, APP_{751} and APP_{770} [23]; APP_{695} is expressed predominantly in the brain. The APP protein is localized to many membranous structures in the cell, including the cell membrane [24]. APP processing components such as the secretase enzymes may be localized to membrane rafts where APP processing occurs; the dynamic nature of membrane rafts plays a pivotal role in the production of toxic vs. non-toxic forms of APP derivatives. APP production can be greatly influenced by cholesterol levels because increased cholesterol can stimulate membrane raft interactions increasing toxic Aβ synthesis [25]. For example, since β-secretase is found in higher abundance in membrane rafts, increased raft interactions favours production of Aβ [26].

Proteolytic processing of APP occurs *via* endocytic and secretory pathways in the membrane of the endoplasmic reticulum (ER) and the trans-Golgi apparatus [27]. In the endocytic pathway full-length APP molecules are internalized and degraded in the endosomal-lysosomal compartment. Internalization occurs by means of a consensus motif, NPTY, between the 759 and 762 regions into clathrin-coated vesicles [28]. Once in lysosomes, APP is processed into amyloidogenic C-terminal proteolytic fragments, which includes Aβ [29]. In contrast, the secretory pathway, which accounts for the majority of APP processing, occurs in the cell membrane; APP is processed either through the amyloidogenic or non-amyloidogenic pathways. Here, APP is proteolytically cleaved by α-, β-, and γ-secretases producing fragments of various sizes [21]. The APP_{770} variant illustrates these amyloidogenic and non-amyloidogenic proteolytic processes (Fig. **1b**). The α- and γ- secretases cleave APP through the non-amyloidogenic pathway, producing αAPPs and p3 fragments. In AD, APP proteolytic processing shifts, becoming more amyloidogenic; this occurs through enhanced β- and γ-secretase activity which gives rise to the neurotoxic fragments, C99 and $A\beta_{1\text{-}42}$ [21, 30]. The β-secretase or β-site APP cleaving enzyme (BACE-1) cleaves APP at the Methionine 671 site, resulting in βAPPs and a C99 fragment. The C99 fragment, which includes the Aβ region is then heterologously cleaved by γ-secretase between Valine 711 and Threonine 714 producing Aβ fragments of size ranging from 40-43 amino acids [1]. People with FAD have mutations in the α-, β- and γ-secretase cleavage sites of the APP gene these mutations are known as the Dutch, Swedish, and London mutations respectively [4]. These mutations present in FAD individuals result in increased production of $A\beta_{1\text{-}42}$, forming plaques in the brain. These observations have been demonstrated using both *in vitro* and *in vivo* models of FAD [31]. Amyloid plaque deposits have also been observed in Down syndrome (DS) individuals. DS, also called trisomy 21 is caused when individuals gain an extra copy of chromosome 21 during meiosis. DS individuals often display symptoms of mild to severe mental retardation and are often recognized by their unique set of physical

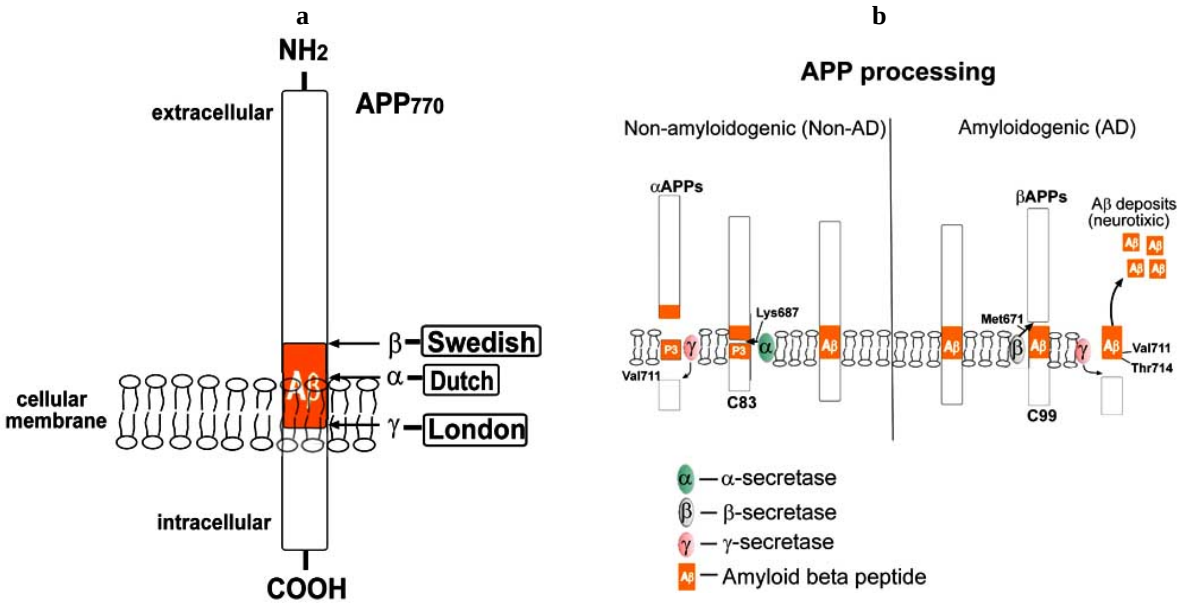

Fig. (1). Schematic representation of APP$_{770}$. (**a**) APP is a transmembrane protein, which has an amino (NH2) terminal on the extracellular end and a carboxyl (COOH) terminal on the intracellular end. The location of the Aβ fragment is within the transmembrane region. APP is cleaved by at various sites by α-, β- and γ-secretases. Mutations in APP are located at cleavage sites referred to as Dutch, Swedish, and London and lead to enhanced Aβ production; they and are associated with the early onset form of AD. (**b**) APP proteolytic processing occurs through either the non-amyloidogenic or amyloidogenic pathway. In the non-amyloidogenic pathway, α-secretase and γ-secretase cleave APP resulting in αAPPs and C83 fragment production. The C83 fragment is then cleaved by γ-secretase to form a 3kD fragment, called p3. The amyloidogenic processing mode, involves β and γ-secretases. β-secretase cleaves the APP, into βAPPs and C99. This C99 fragment is then cleaved to Aβ fragments of various sizes of 40-43 amino acids by γ-secretase.

characteristics. An extra copy of chromosome 21 containing the APP gene results in the overproduction of APP and subsequent build up of Aβ. The age of onset of AD-like symptoms in DS is usually much earlier than in FAD patients. Dementia in DS individuals begins at around 35 years of age and AD-like pathology is usually observed by about 40 years of age [32].

In non-amyloidogenic processing, α-secretase cleaves APP at the Lysine 687 site, which is located on the Aβ fragment region at the NH2 terminal region. The resulting cleaved fragment is a soluble αAPPs, and a C-terminal fragment (C83). The C83 fragment located inside the membrane is next cleaved by γ-secretase at the Valine 711 site, producing a 3 kDa fragment, known as p3 [1]. The non-amyloidogenic pathway producing αAPPs is neuroprotective [33].

CHOLESTEROL, APOE, 24-OHO AND THEIR ROLE IN AD

Cholesterols are amphipathic lipids; they are hydrophobic on one end and hydrophilic on the other. Cholesterol is an essential constituent of cellular membranes contributing to membrane fluidity and effecting proteolytic processing of many membrane based proteins such as APP. Moreover, it is also an important precursor for steroid hormones which act as neurotransmitters which are involved in a plethora of physiological functions. Brain cholesterol accounts for roughly 25% of total body cholesterol [34,35]; the vast majority of brain cholesterol is used in the production of myelin. The myelin sheath, which is manufactured by oligodendrocytes, insulates axons, promoting efficient saltatory conduction of electrical impulses. In humans, cholesterol is obtained by either *de novo* synthesis in the endoplasmic reticulum, or through dietary intake. Since cholesterol does not freely transverse the blood brain barrier (BBB), brain cholesterol is obtained primarily through the *de novo* synthesis pathway [36].

Cholesterol biosynthesis in the brain takes place though a multi-step process involving the enzymes, HMG-CoA reductase, Squaline epoxidase, and Δ7dihydrocholesterol reductase [36]. Molecules of Acetyl Coenzyme-A (Co-A) are converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by HMG-CoA synthase. Next, HMG-CoA is converted to Mevalonate by HMG-CoA reductase. Statins inhibit the HMG-CoA reductase enzyme at this stage, blocking cholesterol biosynthesis. Mevalonate is phosphorylated into Isopentenyl-5-pyrophosphate (IPP). IPP is then converted to squalene by squalene synthase which may also be inhibited by some statins such as Squalastatin. Squalene is oxidized in a couple of steps to Lanosterol by Squalene monooxygenase and Lanosterol synthase. From here, Lanosterol is further modified in numerous steps and finally, converted to Cholesterol (Fig. **2**) [36].

Cholesterol transport in the brain is accomplished primarily by the lipoprotein transporter, apoplipoprotein E (APOE). APOE mediates the uptake and redistribution of lipids in the CNS, through LDL receptors and the lipoprotein receptor related protein (LRP). In the CNS, APOE along with its isoforms plays an important role in cholesterol metabolism. The gene for the cholesterol transport protein, APOE is localized to chromosome 19q13.2. There are three main isoforms of APOE: ε2, ε3, and ε 4. Isoforms ε2 and ε3 are considered protective while the presence of the APOEε4 allele is a ge-

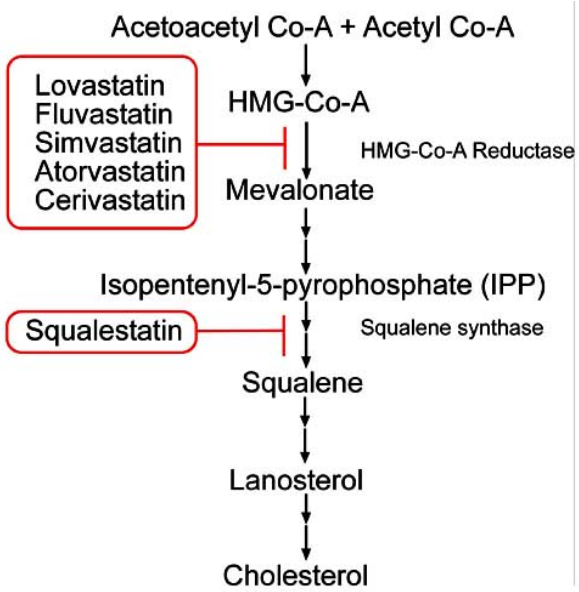

Fig. (2). Statins interfere with cholesterol biosynthesis. Statins prevent cholesterol biosynthesis by inhibiting the activity of two key enzymes, HMG Co-A reductase and squalene synthase.

netic risk factor for both sporadic forms and familial forms of AD [6, 37, 38]. Susceptible individuals possessing one allele of APOEε4 develop AD approximately 5-10 years later than those individuals with both copies of the allele [39]. The low-density lipoprotein receptor related protein (LRP) and the very low-density lipoprotein receptor are the primary receptors for APOE in the brain and have been linked to APP processing [40,41]. The LRP pathway has been shown to have a role in APOEε4 induced production of Aβ [42]. This was demonstrated by blocking the LRP pathway using the inhibitor, receptor-associated protein (RAP). Inhibition of the LRP using RAP to reduce APOEε4, enhanced Aβ production. According to the study, APOEε4 interacts directly with LRP to alter APP signaling. Higher levels of Aβ have been observed in AD patients possessing the APOEε4 allele [43]. An elevated level of APOEε4 in Tg2576 mice also correlates with increased plaque loads and formation of cerebral amyloid angiopathy. These results suggest a direct role for APOEε4 in APP processing. In addition, an effect on clearance was noted as it was found that APOEε4 decreased $A\beta_{1\text{-}40:42}$ ratios in the CSF and increased $A\beta_{1\text{-}40:42}$ ratios in brain extracellular pools [44].

Cholesterol in the brain is removed as oxysterol, 24S-hydroxysterol (24-OHO) by the enzyme 24S-hydroxylase (CYP46). 24-OHO can cross the BBB, enter the plasma and be delivered to the liver for excretion as bile [40]. The half-life of cholesterol in the brain is approximately six months, which is relatively long, compared to cholesterol turnover rates in other organs. This slow conversion rate of cholesterol to 24-OHO allows for its use as a biomarker for assessing cholesterol turnover in the brain. Increased levels of 24-OHO have been observed in the plasma and CSF of AD patients and may be an indication of altered cholesterol metabolism in these individuals. Patients showing symptoms of AD showed alteration in the levels of brain oxysterols when compared to normal brains [45]. In a related study, AD patients and individuals with vascular dementia displayed significantly higher concentrations of 24-OHO than healthy controls. However, it has been suggested that the higher level of 24-OHO observed in AD patients may be a by-product of recycled cholesterol from dying neurons. 24-OHO levels have been shown to increase with mild to moderate AD and decrease with advanced AD, likely due to increased neuronal loss over time. Moreover, a loss of neurons means a loss in 24-OHC, which may result in a build-up of cholesterol in the brain and less 24-OHO clearance [45]. Further human studies are required to follow the levels of 24-OHO and cholesterol with advancing stages of AD from diagnosis of mild to severe forms in order to appropriately assess the efficacy of 24-OHO for use as a biomarker in AD.

ROLE OF STATINS IN AD

While the main indication for statins remains hypercholesterolemia there is promise of new uses for this class of drugs. Early epidemiological studies showed the existence of a link between statin use and a decreased prevalence of AD, there has been much attention focused on confirming these findings; more specifically, elucidating the mechanism(s) involved in neuroprotection conferred by statins. In the past 8 years, there have been mixed results from epidemiological studies however, this may be due to factors such as: patient inclusion criteria, study duration, statin dosage, and end-points etc. In spite of conflicting reports, there is increasing evidence for the efficacy of statin use in AD from molecular based studies.

$A\beta_{1\text{-}42}$ is neurotoxic and pro-apoptotic, thus reduction in $A\beta_{1\text{-}42}$ levels may in fact be one of the mechanisms by which statins reduce AD related dementia. Statins have been shown to reduce the levels of Aβ *in vitro*, in animals and in humans. Primary cultures of mice hippocampal and cortical neurons treated with simvastatin and lovastatin reduced production of both intracellular and extracellular Aβ. Similar effects *in vivo* were also found using guinea pigs treated with high doses of simvastatin, there was a significant reduction in $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels [46]. Results from *in vitro* and *in vivo* studies were also observed in a study using human subjects. In a double-blind, randomized, placebo controlled human study, participants were treated with 10, 20, 40 or 60 mg once-daily doses of lovastatin; patients receiving lovastatin displayed a dose dependent decrease in serum Aβ levels which were significantly reduced from that of controls at 40 and 60mg doses [47]. Several hypotheses have been proposed to explain how the reduction in Aβ levels by statins occurs. α-secretase activity, which is neuroprotective, has been implicated as one of the components responsible for Aβ reduction. SH-SY5Y human neuroblastoma cells exposed to $A\beta_{1\text{-}42}$ showed a significant increase in the level of caspase-3, an apoptotic enzyme, and a decrease in the APP processing enzyme, α-secreatase. Rosuvastatin mitigated these effects by reducing the expression of caspase-3 and up-regulating α-secretase activity, thereby promoting neuroprotection [48]. Cleavage of APP by α-secretase results in the production of sAPPα fragment. sAPPα stimulates neurite outgrowth and synaptogenesis [49-51]. In addition, sAPPα reduces the toxic effects of glutamate and Aβ peptide protecting hippocampal and cortical neurons [50]. Treatment with lovastatin has been shown to increase α-secretase levels, and sAPPα [49].

A reduction in β-secretase activity is also reported to be involved in amyloid processing disturbances triggered by statins. Work by Parsons *et al*., 2006, showed that statins may interfere with the dimerization of β-secretase thus, lowering its activity and reducing the amounts of Aβ produced. However, a linear dose-response was not observed. In fact, using ELISA, it was shown that Aβ release was higher with 50 μM simvastatin than with 1 μM simvastatin in HEK-293 cells in spite of both doses showing positive effects by reducing Aβ production. This may mean that lower doses of statins may be more effective at treating AD. Further studies are required to explore this phenomenon as statins may have dose-dependent effects on various pathways becoming activated; statin dose may dictate whether protection it is cholesterol dependant or independent [52].

Finally, γ-secretase activity, which is well established as being responsible for Aβ production, can also be regulated by statins. γ-Secretase activity is increased in the presence of isoprenoids such as geranylgeranyl pyrophosphates, which are more abundant when cholesterol levels are high. Lovastatin and simvistatin were both able to reduce cholesterol levels in HEK293 cells with a concomitant reduction in geranylgeranyl pyrophosphate levels; $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels were also reduced. A block in Aβ production by statins was overcome by treatment of geranylgeraniol, an isoprenoid equivalent [53].

Apart from secretase activity, statins also decrease Aβ production through inhibition of protein isoprenylation of Rho and Rab, members of the G-protein family [54]. Rab1b and Rab6 are critical for vesicular trafficking and are involved in Aβ production. Rab1b promotes the export of APP from the endoplasmic reticulum to the Golgi. Inhibition of Rab1b using dominant-negative mutants led to a 90% reduction in Aβ secretion. Rab6 is involved in intra-golgi trafficking of APP; inhibition of its function using dominant-negative Rab6 mutants also led to a significant decrease in Aβ generation [55]. Under normal conditions, Rho and Rab require the addition of isoprenyl moieties at their C-termini to elicit GTPase activity. Statins function by lowering the concentration of isoprenyl intermediates such as, geranylgeranyl pyrophosphate and farnesyl pyrophosphate. Therapeutic doses of simvastatin and lovastatin (200 nM) have reduced APP C-terminal and Aβ fragments by effecting GTPase activity [54]. Moreover, since abundant Aβ production occurs on membrane rafts and treatment with statins has been shown to decrease the density of membrane rafts *via* a reduction in cholesterol; statin treatment should presumably have a lowering effect on Aβ production [25].

Besides decreasing Aβ production, statins also have other pleiotropic effects. These include anti-inflammation, immunomodulation, and vasoactive effects. Inflammation in the AD brain occurs as a result of plaque deposition. Inflammatory mediators from cells such as microglia and astrocytes, and molecules like IL-1 and IL-6 have been detected, localized to senile plaques, in the AD brain. The induction of a microglial-driven inflammatory response results in the release of a wide-array of neurotoxic cytokines. Activation of the complement cascade and initiation of inflammatory enzyme systems such as inducible nitric oxide synthase (iNOS) and the prostanoid generating cyclooxygenase-2 (COX-2) enzyme, have also been implicated [56]. Statins exert anti-inflammatory effects by regulating pro-inflammatory molecules such as: inducible nitric oxide synthase (iNOS), Interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) in astrocytes and macrophages [57]. Increased levels of pro-inflammatory gene products like cyclooxygenase-2 (COX-2) and cytosolic phospholipase-A2 are observed in human neurons and mouse primary mixed neuronal and glial cultures treated with 24-OHO; the increase in inflammatory mediators was partially suppressed by simvastatin treatment [19]. Human glioma cells pre-treated with pravastatin and then exposed to Aβ displayed lower metalloproteinases (MMPs), IL-6, and free radical expression than Aβ alone [58]. Separately, both simvastatin and atorvastatin have been shown to alleviate inflammation in mice after traumatic brain injury. Statins were shown to reduce the expression of inflammatory cytokines, and interfere with leukocyte recruitment and migration to the CNS. Additionally, treatment with statins also improved behavioral outcome in these mice [59].

The anti-inflammatory mechanism of statins is distinct from its cholesterol reducing properties. The Ras and Ras-like G-binding proteins: Rac, Ral, Rap and Rho require isoprenylatation for carrying out normal function, which includes cell proliferation, migration and signaling. Statin treatment causes the intracellular accumulation of inactive Rho through inhibition of the Rho kinase pathway. Addition of isoprenyl precursors, mevalonic acid, and geranylgeranyl pyrophosphate (GGpp) reversed the statin-mediated down-regulation of inflammatory proteins [60]. The anti-inflammatory effects of statins also work in conjunction with their immunomodulatory properties. Aβ and interferon-gamma (IFN-γ), which are known to activate the Janus kinase (JAK)/ signal transducer and activator of transcription (STAT) pathway, induces microglial CD40 expression in cultured microglial cells; CD40 prevents the microglial phagocytosis of Aβ. Treatment with lovastatin prevented IFN-γ-induced expression and promoted the phagocytosis of Aβ [61]. Another pro-inflammatory pathway involving advanced glycation end products (AGE) has also shown benefits from statin treatment. AGEs are products of non-enzymatic reactions with amino groups of proteins. Protein deposits observed in AD show Aβ plaques and tangles are cross-linked to AGE. Glia, which are activated by AGE, secrete a broad range of pro-inflammatory molecules that are associated with AD. These include: NO, IL-1β, IL-6, TNF-α, and macrophage colony stimulating factor [62]. AGEs also bind to the signal transducing receptor, RAGE, inducing oxidative stress and inflammation. Statins reduce AGE levels, independent of their cholesterol lowering effect. Atorvastatin was shown to significantly reduce AGE levels in hypercholesterolemic type-2 diabetic patients [63]. Future studies may find that statin use leads to a reduction in AGE and thus, may interfere with plaque deposition.

Vasoconstriction of the cerebral vessels is yet another feature of AD. Aβ peptides form deposits in the cerebrovasculature which can result in cerebral amyloid angiopathy (CAA) and hemorrhagic stroke [64]. Transgenic AD mice (Tg2576), show altered angiogenesis and reduction in vascular density in the cortex and hippocampus compared to littermate controls [65]. Using a Doppler imager, one study showed a progressive decline in cortical perfusion levels in Tg2576 mice when compared to age-matched control littermates [64]. The vasoconstrictive effect of Aβ was also dem-

onstrated using isolated porcine cerebral basilar arteries. In this study, $A\beta_{1-40}$ prevented the stimulation of sympathetic alpha7-nicotinic acetylcholine receptors (α7-nAChRs), which cause the release of nitric oxide (NO) in parasympathetic nitrergic nerves and subsequent vasodilatation. This vasocontrictive effect of $A\beta_{1-40}$ on the cerebral arteries was reversed on treatment with HMG-CoA inhibitors, mevastatin and lovastatin [66]. Similarly, in humans, Aβ in association with endothelin-1 (ET-1) induces vasoconstriction. This has been demonstrated in isolated human cerebral middle and basilar arteries [64]. Interestingly, vasoconstriction was completely antagonized by a cyclooxygenase-2 kinase inhibitor, NS-398 [64]. Since statins have been shown to decrease COX-2 expression [67], future studies may confirm a link between vasodilation and neuroprotection through this mechanism leading to a decreased incidence of AD.

Many other pleiotropic effects of statins for use in AD treatment and prevention have been suggested. Addition of pravastatin to hippocampal neurons significantly increased neurite length and branching suggesting that statins are involved in regeneration. Moreover, this effect was suggested to be dependent on statin mediated isoprenyl inhibition. More specifically, geranylgeranylation inhibition, but not farnesylation, mimicked the stimulatory effect of pravastatin on neurite outgrowth [68].

ANTI-APOPTOTIC SIGNALING PATHWAYS OF STATINS IN AD

The cause of neuronal loss in AD is primarily due to apoptosis or programmed cell death. Hallmarks of apoptosis include: cell blebbing, chromatin condensation and nuclear fragmentation. A fine balance between pro-apoptotic and anti-apoptotic molecules is required in order to maintain homeostasis, however, deviation during pathological processes can shift the balance in favor of cell death. $A\beta_{1-42}$ has been shown to increase the expression of Bax and caspase-3 with a concomitant decrease in Bcl-2 expression in IMR-32 human neuroblastoma cells [69].

Statins have demonstrated their anti-apoptotic effects by blocking $A\beta_{1-42}$ induced neuronal death. Statins modulate the expression of molecules involved in the apoptotic process. Expression of the pro-survival molecule, Bcl-2 is up-regulated in neurons both *in vitro* and *in vivo* upon treatment with simvistatin. In the same study, pre-treatment with simvistatin reduced caspase-3 activity and cell death in neurons exposed to $A\beta_{1-42.}$ However, when G3139, an anti-sense oligonucleotide directed against Bcl-2, was used, the protective effects of simvistatin were abolished suggesting Bcl-2-dependent protection [70, 71]. Depending upon the degree of neurodegeneration, statin treatment may benefit individuals in both early and middle stages of AD. Treatment of late-stage AD using statins, however, may not be as successful since there is often widespread neurodegeneration at this point. Studies have shown that increased levels of $A\beta_{1-42}$ in the advanced stages of AD tilt the homeostatic balance causing a down-regulation of Bcl-2 and increased expression of Bax [72]. Future research could test the validity of using statins at various stages of AD progression. Another statin, rosuvastatin has also been shown to exhibit anti-apoptotic actions in SH-SY5Y cells treated with $A\beta_{1-42}$ by decreasing caspase-3 activity, approximately 48%; statin mediated up-regulation of α-secretase was also noted, which increases the pro-survival fragment, sAPP-α [48].

Statins have also been demonstrated to activate and up-regulate the expression of anti-apoptotic pathways (Fig. **3**). It is likely that statins could potentiate the activation of many pro-survival pathways, however, the activation of only a few pro-survival pathways have been demonstrated in neuronal systems [73]. Other pro-survival activation has been demonstrated using cardiac and stem cells [74].

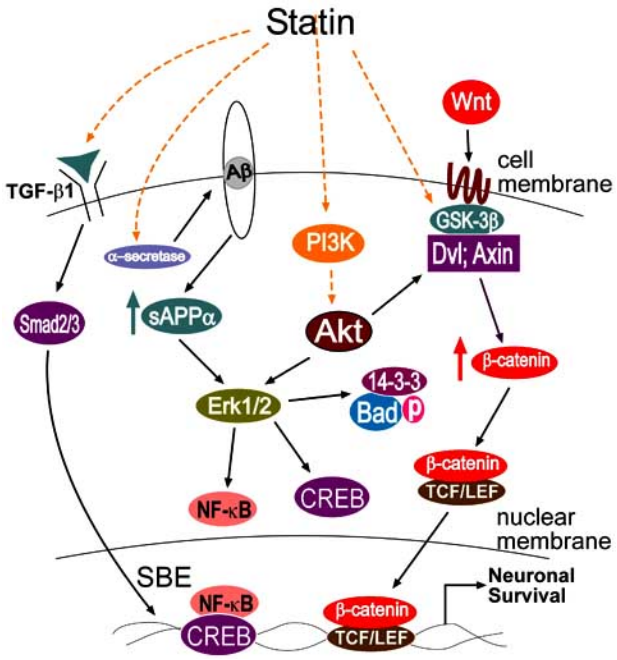

Fig. (3). Anti-apoptotic pathways activated by statins. Statins inhibit the activity of GSK-3β, up-regulating TCF/LEF-β-catenin, and promoting neuronal survival. APP processing is also affected by statin treatment leading to increased sAPPα, which is neuroprotective. TGF-β1 signaling is also activated on statin treatment promoting neuronal survival. Statins can also activate Erk 1/2 *via* PI3K and Akt. Active Erk 1/2 can phosphorylate and thus, lead to inactivation of Bad. Furthermore, ERK 1/2 can activate NF-κB and CREB therefore, allowing transcription of genes which promote neuronal survival.

Wnt signaling is neuroprotective against the toxic effects of Aβ induced neurotoxicity [73]. Wnt's are secreted cysteine-rich glycoproteins that bind to frizzled receptors and mediate cell-signaling [75]. When there is a lack of Wnt ligands, β-catenin is led to a destruction complex that includes adenomatous polyposis coli (APC), Axin and active glycogen synthase kinase-3β (GSK-3β). However, in the presence of Wnt ligands, the bound frizzled receptor activates the phospholipoprotein, dishevelled, (Dvl). Axin, a negative regulator of Wnt signaling, releases β-catenin and permits it's translocation to the nucleus. Additionally, the activity of GSK-3β is inhibited. The resulting increase in β-catenin expression leads to complex formation with TCF/LEF transcription factors, resulting in the expression of Wnt related genes, some of which are involved in neuronal survival [75]. In a study demonstrating the neuroprotective role of β-catenin-TCF/LEF signaling, transfection of dominant-negative TCF construct incorporated into mice hippo-

campal neurons, increased susceptibility to Aβ-induced toxicity [76]. Separately, Aβ treatment caused a decrease in cytosolic β-catenin levels and led to neuronal apoptosis [76]. Aβ activates GSK-3β, which degrades β-catenin and decreases expression of pro-survival mediators [77]. Active GSK-3β has been observed in AD brain regions alongside neurofibrillary tangles (NFTs); this implies that the phosphorylation of Tau proteins by active GSK-3β may be associated with reduced pro-survival Wnt signaling, in AD brain [78].

Increased Wnt signaling might be beneficial to the neurons in the brain for survival. A number of alternatives are being explored towards increasing β-catenin expression to promote Wnt signaling [73]. In our study we showed that statins act by blocking the activity and expression of GSK-3β, thereby increasing β-catenin levels. Lithium (Li) can inhibit GSK-3β activity and has been shown promote neuronal survival by increasing the levels of β-catenin [77]; it is likely that lovastatin used in the study acts akin to Li. Although, the mechanism of statin mediated neuronal survival is yet to be determined. It is possible that the mode of action is direct, where statins act by competitively inhibiting Mg^{++} binding to the active site of the enzyme. Alternatively, the action could be indirect, where activation of pro-survival pathways such as PI3K/Akt and MEK/ERK pathways could inhibit the activity of GSK-3β [79].

Akt acts on a number of targets allowing cells to survive. The Akt pathway is activated by Phosphoinositide 3-kinase (PI3K) *via* phosphorylation. The most prominent cell survival action sites of Akt are NF-κB, cAMP-response element binding protein (CREB) and Bad, all of which are involved in regulating cell survival [80]. Simvastatin has been shown to enhance retinal ganglion cell (RGC) survival by increasing Akt phosphorylation *in vivo*, suggesting that the PI3K/Akt pathway may contribute to its protective role in the CNS [81]. Future studies could investigate the role of statins in promoting neuronal survival with $A\beta_{1\text{-}42}$ treatment *via* Akt activation.

Activation of Mitogen activated protein kinases (MAPK) also play an important part in neuronal survival. Extracellular signal-regulated kinase 1/2 (Erk 1/2) in particularl, is known to be a pro-survival MAPK. MAPK activate the cell survival mechanism by activating pp90 ribosomal S6 kinase (RSK). RSK in turn phosphorylates Bad, inhibiting its pro-apoptotic activity, and activates CREB, which activates the transcription of Bcl-2; Bcl-2 promotes neuronal survival [80]. Lovastatin has been shown to protect mesenchymal stem cells against hypoxia and serum deprivation-induced apoptosis by activation of PI3K/ Akt and Erk 1/2. The role of Erk in mediating cell survival was substantiated using inhibitor, U0126. Separately, this study also showed that statins block the activation of GSK-3β, protecting mesenchymal stem cells [82]. Future studies could expand on this research using an AD model and examine the role of statins in activating Erk 1/2 in neurons treated with $A\beta_{1\text{-}42}$, to examine neuronal survival.

TGF-β1 is a multifunctional cytokine that is capable of regulating diverse cellular processes including neuronal survival and apoptosis. TGF-β1 signaling involves the binding of the ligand to the TGF-β1 receptor promoting the co-localization of Smad2 and Smad3 with Smad4 leading to the activation of Smad binding elements (SBE). Alternatively, inhibition *via* inhibitory Smad7 leads to the block of TGF-β1 signaling. Activation of TGF-β1 signaling in rat hippocampal cells led to activation of Erk 1/2 and Rsk1, phosphorylating Bad, and prevention of apoptosis. Alternatively, TGF-β1 signaling can lead to neuronal apoptosis in AD due to $A\beta_{1\text{-}42}$ toxicity. In our study, we showed that Smad7 interaction with β-catenin, under $A\beta_{1\text{-}42}$ toxicity leads to apoptosis [83]. A recent study has shown that statins promote TGF-β1 signaling by activating Smad2 and Smad3, leading to cell survival. It would be interesting to see if there is an alteration in TGF-β1 signaling during co-treatment with statin and $A\beta_{1\text{-}42}$.

PKC activation is associated with cell survival, and has been shown to protect neurons from Aβ-induced apoptosis [84, 85]. Atorvastatin has been shown to have an anti-apoptotic effect in rat cardiomyocytes, through the activation of PKC. PKC anti-apoptotic effect was inhibited using inhibitors, rottlerin and chelerythrine, but this was ablated using atorvastatin. Moreover, caspase-3 activity was also reduced in cardiomyocytes treated with atorvastatin [86]. Further studies are required to ascertain whether PKC is activated in neurons co-treated with $A\beta_{1\text{-}42}$ and statins.

Mutations in APP are a significant cause for increased $A\beta_{1\text{-}42}$ production, leading to neuronal apoptosis [87]. Mutant APP has been shown to result in aberrant signaling, causing staurosporine treated cells to be more sensitive to insult, leading to neuronal apoptosis [88, 89]. Research has shown that statins reduce $A\beta_{1\text{-}42}$ levels [46], the prime cause of neuronal toxicity. Transfected wild-type APP in rat neuroblastoma (B103) cells has been used to demonstrate protection from inducers of apoptosis [82]. It appears that the presence of wild-type APP was responsible for blocking both p53 DNA binding and transactivation therefore, preventing apoptosis. B103 cells transfected with FAD mutant APP were prone death by this mechanism. Future research could examine the role of statins in improving neuronal survival in cells transfected with the mutAPP gene. Addressing this issue may be of significance because individuals possessing the APPmut gene have a higher probability of developing AD. In this regard, the role of other apoptotic mediators such as: LSF, p38/MEF2, p53 and Rab5 could also be examined in APP deficient cells transfected with the APPmut [88-91]. More specifically, it would be interesting to see if APPmut transfected cells could be rescued *via* treatment by statins.

CONCLUSION

Statins are HMG-CoA inhibitors that are indicated for patients with cardiovascular problems, to reduce LDL cholesterol levels. Statins are beginning to be investigated as a therapeutic option for AD treatment. A clinical trials correlation with cardiovascular patients first showed the potential for statins in reducing the incidence of AD.

Statins not only lower cholesterol but they also reduce neurotoxic Aβ by altering APP processing therefore, promoting neuronal survival. Molecular-based studies have also shown that statins have pleiotropic effects which include: anti-inflammation, immunomodulation and vasoactive effects.

Anti-apoptotic signaling pathways are activated with statin treatment reducing the expression of caspase-3. The pro-survival signaling pathways that are stimulated upon

statin exposure are only beginning to be explored in AD models. In our own study, we demonstrated the neuroprotective role of β-catenin-TCF/LEF against Aβ-toxicity with statin treatment. Pro-survival signaling pathways such as PI3K, Akt and Erk (MAPK) have also been demonstrated to become activated with statin treatment. Future studies should examine the role of these anti-apoptotic pathways in AD treatment.

AD is a chronic condition therefore, statin therapy for an indefinite period of time maybe required for its efficacy to be maintained. The long-term benefits of statin use and the effects of statin withdrawal would have to be assessed for patients with AD. Future research should also elucidate the mechanism(s) by which statins reduce cleaved APP products through isoprenylation of G-proteins. A thorough understanding of this mechanism may lead to the development of novel therapeutics which specifically target the G-proteins involved with Aβ production and which may lead to an overall reduction in Aβ deposition.

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Received: February 06, 2009 Revised: April 04, 2010 Accepted: April 04, 2010