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Isoprenoids and Related Pharmacological Interventions: Potential Application in Alzheimer's Disease

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Abstract

Two major isoprenoids, farnesyl pyrophosphate and geranylgeranyl pyrophosphate, serve as lipid donors for the posttranslational modification (known as prenylation) of proteins that possess a characteristic C-terminal motif. The prenylation reaction is catalyzed by prenyltransferases. The lipid prenyl group facilitates to anchor the proteins in cell membranes and mediates protein-protein interactions. A variety of important intracellular proteins undergo prenylation, including almost all members of small GTPase superfamilies as well as heterotrimeric G protein subunits and nuclear lamins. These prenylated proteins are involved in regulating a wide range of cell processes and functions, such as cell growth, differentiation, cytoskeletal organization and vesicle trafficking. Prenylated proteins are also implicated in the pathogenesis of different types of diseases. Consequently, isoprenoids and/or prenyltransferases have emerged as attractive therapeutic targets for combating various disorders. This review attempts to summarize the pharmacological agents currently available or under development that control isoprenoid availability and/or the process of prenylation, mainly focusing on statins, bisphosphonates, and prenyltransferase inhibitors. Whereas statins and bisphosphonates deplete the production of isoprenoids by inhibiting the activity of upstream enzymes, prenyltransferase inhibitors directly block the prenylation of proteins. As the importance of isoprenoids and prenylated proteins in health and disease continues to emerge, the therapeutic potential of these pharmacological agents has expanded across multiple disciplines. This review mainly discusses their potential application in Alzheimer's disease.

Keywords

Isoprenoids; Protein Prenylation; Statins; Bisphosphonates; Prenyltransferase Inhibitors; Alzheimer's Disease

INTRODUCTION

Isoprenoids are short-chain lipid molecules produced in the mevalonate pathway (Fig. 1) (Goldstein and Brown, 1990). They include isopentenyl pyrophosphate (IPP), farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). FPP and GGPP serve as

lipid donors for the posttranslational modification (known as prenylation) of proteins that possess a characteristic C-terminal motif, CAAX, in which a cysteine (C) residue is followed by two small aliphatic (A) residues and a variable (X) residue. Prenylation occurs at the C residue with the attachment of either farnesyl or geranylgeranyl group catalyzed by prenyltransferases: farnesyl transferase (FT) and geranylgeranyl transferase type I (GGT-I) and II (GGT-II or RabGGT). The X residue contributes significantly to the specificity of prenyltransferases (Lane and Beese, 2006). The lipid prenyl group facilitates to anchor the proteins in cell membranes and mediates protein-protein interactions. A variety of important intracellular proteins including heterotrimeric G protein subunits and nuclear lamins are prenylated but the largest, and most extensively studied group, is the small GTPase superfamilies including the well-known Ras, Rab, Rho and Rac (McTaggart, 2006). Small GTPases are essential signaling proteins that regulate a variety of cell processes and functions, such as cell growth, differentiation, cytoskeletal organization and vesicle trafficking. The prenylation status of small GTPases affects their intracellular trafficking, subcellular localization and interactions with substrates. Therefore, prenylation modifies the functions of small GTPases as well as the functions of their downstream effectors. Prenylated proteins have been found to play a critical role in the development of some cancers and other diseases including cardiovascular and cerebrovascular diseases, bone diseases, Alzheimer's disease (AD), and progeria. Consequently, isoprenoids and/or prenyltransferases have emerged as attractive therapeutic targets. This review attempts to summarize the pharmacological agents currently available or under development that control isoprenoid availability and/or the process of prenylation, i.e., statins, bisphosphonates, and prenyltransferase inhibitors, and their potential therapeutic applications, focusing on Alzheimer's disease.

STATINS

Statins are a class of drugs that have been successfully used for the treatment of hypercholesterolemia and the prevention of coronary heart disease in the last 20 years (4S-group, 1994; Sacks et al., 1996). Statins selectively inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate limiting enzyme of the mevalonate pathway (Fig. 1) (Goldstein and Brown, 1990), which is the principal cholesterol biosynthesis pathway in eukaryotic cells. By targeting HMG-CoA reductase, statins block the conversion of HMG-CoA into mevalonate, the initiating step of cholesterol biosynthesis, and thus lower the production of downstream molecules including isoprenoids and cholesterol. In the liver, the reduced intracellular cholesterol level induces the up-regulation of LDL (low-density lipoprotein) receptors that thereby restore the intracellular cholesterol homeostasis, which subsequently promotes the clearance of cholesterol-loaded LDL in the blood (Brown and Goldstein, 1986).

Several statins have been approved by the US Food and Drug Administration (FDA) (Table 1). These statins vary in their derivation, physical properties, pharmacology, and pharmacokinetics. Lovastatin is a natural product of a fungus *Aspergillus terreus*, whereas others are produced by chemical modification of lovastatin (such as simvastatin and pravastatin) or by chemical synthesis (such as atorvastatin, fluvastatin, rosuvastatin, and pitavastatin) (Jones, 2003; Shitara and Sugiyama, 2006). Each of the statins has a

characteristic structure that interacts with the binding site of HMG-CoA reductase (Istvan and Deisenhofer, 2001), inhibiting the enzyme activity and the production of downstream molecules. Although all statins share a common mechanism for inhibiting HMG-CoA reductase, they differ in terms of their potency and their ability to cross the blood-brain barrier (BBB) (Table 1) (Jones, 2003; Schachter, 2005; Shitara and Sugiyama, 2006; Yee and Wright, 2011).

A large body of evidence indicates that the beneficial effects of statins in preventing coronary heart disease extend beyond their cholesterol-lowering ability (Liao, 2002). Cholesterol-independent (pleiotropic) effects of statins have been shown in a number of *in vitro*, animal models and clinical studies, such as reducing the risk of stroke (Vaughan, 2003), osteoporosis (Meier et al., 2000), and multiple sclerosis (Vollmer et al., 2004). Many of the pleiotropic effects of statins are shown to be mediated through inhibition of the synthesis of isoprenoid intermediates, such as FPP and GGPP, in the mevalonate pathway (Liao, 2002; Vaughan, 2003) (Fig. 1).

More recently, epidemiological studies show that statins decrease the risk of AD although prospective studies have produced mixed results. The discrepancies among these studies most likely are caused by differences in the choice and dose of statins, the selection of patient populations, the treatment durations, and the criteria of outcome measures (recently reviewed by (Shepardson et al., 2010b; Shepardson et al., 2010a)). Since epidemiological studies have demonstrated a correlation between high cholesterol levels and the incidence of AD (Jarvik et al., 1995; Pappolla et al., 2003; Fernandez Martinez et al., 2008), some of the benefits of statins in AD could be attributed to the well-established cholesterol-lowering function of statins. Indeed, several *in vitro* and *in vivo* studies have shown that statins modulate the processing of amyloid- β precursor protein (APP) and decrease the production of amyloid- β peptide (A β) through lowering the cellular cholesterol content (Simons et al., 1998; Fassbender et al., 2001; Kojro et al., 2001). However, emerging evidence indicates that beneficial effects of statins in AD are not limited to lowering levels of cholesterol. The isoprenoid-dependent pleiotropic effects of statins may also contribute to their role in AD therapy as summarized below.

Effects on APP/A β metabolism

One of the pathological hallmarks of AD is the deposition of aggregated A β in neuritic plaques and cerebral vessels. A β (38–43 amino acids) is derived from a large transmembrane glycoprotein, APP, by proteolytic processing. The generation of intact A β requires the activity of both β -secretase and γ -secretase, which cleave within the luminal and transmembrane domain of APP, respectively. The α -secretase cleaves within the sequence of A β , thus precluding the formation of intact A β (non-amyloidogenic processing of APP), and produces the neurotrophic soluble fragment, sAPP α (Thinakaran and Koo, 2008).

A number of studies have shown that statins affect APP processing and A β production through isoprenoid-dependent pathways as well as cholesterol-dependent pathways. Atorvastatin and simvastatin were found to stimulate α -secretase activity and shedding of non-amyloidogenic sAPP α by depleting FPP and inhibiting farnesylation of Rho and Rho kinase in a murine neuroblastoma cell line (Pedrini et al., 2005). In contrast, other *in vitro*

studies reported that lovastatin or simvastatin augments intracellular accumulation of APP and A β , in parallel with a decrease of secreted A β , in a GGPP-dependent manner (Cole et al., 2005; Ostrowski et al., 2007; Zhou et al., 2008). The mechanisms proposed include the increase/activation of β -secretase (Cole et al., 2005), inhibition of vesicle trafficking (Ostrowski et al., 2007), and inhibition of γ -secretase activity (Zhou et al., 2008). However, a recent study showed that statins decrease A β levels only by lowering cellular cholesterol levels rather than the levels of FPP/GGPP or prenylation in a human neuroblastoma cell line (Hooff et al., 2010). *In vivo*, many studies found no significant changes in the level/deposition of A β with statin treatments (reviewed in (Wolozin et al., 2006)), while the beneficial effects of statins were still observed. We have demonstrated that simvastatin enhances spatial learning and memory without affecting the level/deposition of A β in a transgenic mouse model of AD, Tg2576 mice (Li et al., 2006). A careful comparison of published experimental studies revealed that reduction of A β was only observed in the studies where a high dose of the drug was used and/or the drug treatment was initiated before the manifestation of β -amyloidosis. Yet, a recent study demonstrated that fluvastatin, at clinical doses, significantly reduced the level of A β and the C-terminal fragments (CTFs) of APP in the brain of wild-type C57BL/6 mice (Shinohara et al., 2010). The underlying mechanisms include enhanced trafficking of APP-CTFs from endosomes to lysosomes for degradation, associated with marked decrease of prenylated Rab proteins, and increased clearance of A β from the brain through upregulation of low-density lipoprotein receptor-related protein 1 (LRP-1) at the blood-brain barrier (Shinohara et al., 2010). Clearly, the effects of statin-induced depletion of isoprenoids on APP/A β metabolism are much more complex than previously thought.

Immunomodulatory effects

Convincing data indicate that innate immunity are involved in the pathogenesis of AD (recently reviewed by (Eikelenboom et al., 2011)). Many inflammation-related proteins, such as immunoglobulins, complement factors, α 1-antichymotrypsin, apoE, clusterin, intracellular adhesion molecule-1, α 2-macroglobulin, C-reactive protein, serum amyloid P component and heparan sulphate proteoglycans, have been identified in senile plaques and neurofibrillary tangles (Eikelenboom and Stam, 1982; Abraham et al., 1988; Coria et al., 1988; Bauer et al., 1991). These proteins affect the transport, aggregation, and deposition of A β (Eikelenboom et al., 2011). Conversely, A β activates microglia and astrocytes and upregulates the expression of innate immune receptors and secretion of pro-inflammatory cytokines and other inflammatory mediators (Cameron and Landreth, 2010; Fuller et al., 2010), leading to the activation of the complement system and the initiation of inflammatory cascades. While strong acute immune response could be protective initially, chronic upregulation of certain pro-inflammatory factors could interfere with removal mechanisms of A β , exacerbating AD pathology (Eikelenboom et al., 2011). The importance of immune function in AD is further underscored by findings from recent genome-wide association studies, in which six of the nine new genes that are associated with sporadic AD are also involved in immune functions (Morgan, 2011).

Statins possess anti-inflammatory and immune-modulatory properties. *In vitro* studies have demonstrated that statins regulate pro-inflammatory molecules such as inducible nitric oxide

synthase (iNOS), interleukin-1 β (IL-6), and tumor necrosis factor- α (TNF- α) (Menge et al., 2005). For instance, pravastatin pretreated human glioma cells show lower IL-6 and free radical expression when exposed to A β (Sun et al., 2003). Statins reduce the expression of inflammatory cytokines and interfere with leukocyte migration to the central nerve system (CNS) (Wang et al., 2007). In cultured microglial cells, lovastatin attenuates microglial activation by suppressing the functional expression of CD40, which prevents A β phagocytosis, (Townsend et al., 2004). Lovastatin also inhibits the expression of TNF and IL-1 β (Pahan et al., 1997), and effectively decreases autoimmunity and promotes myelin repair in glial cells (Paintlia et al., 2008). Moreover, microglia cultures exposed to simvastatin and atorvastatin showed reduced level of pro-inflammatory cytokine IL-6 (Lindberg et al., 2005). Recently, it has been shown that simvastatin treatment also prevents A β -induced production of interferon- γ (IFN- γ) and enhances the immune responses to A β vaccination (Kou et al., 2010).

Interestingly, despite the extensive reports on anti-inflammatory effects of statins, the impact of statins on the immune system of the CNS remains elusive. While no mechanism dominates the anti-inflammatory effects of statins, they are partly attributable to the inhibition of small GTPase prenylation. For example, statin-mediated inhibition of Rho GTPase leads to the attenuation of A β deposit-associated inflammation (Cordle et al., 2005). Statin treatments also cause the accumulation of un-prenylated, nonfunctional small GTPases and inflammatory inhibition, which can be reversed by the addition of isoprenyl precursor or GGPP (Cordle and Landreth, 2005).

Neuroprotective effects

Apoptosis-mediated neuron loss represents one of the key detrimental hallmarks in AD pathology. Substantial evidence suggests that statins protect neurons by suppressing A β -induced apoptosis. For instance, simvastatin treatment enhances the expression of Bcl-2, a pro-survival molecule, and reverses the A β induced expression of caspase-3 and neuron death. With the addition of Bcl-2 anti-sense oligonucleotides, however, this neuroprotective effect of simvastatin is abolished, suggesting the protective effect is Bcl-2 dependent (Johnson-Anuna et al., 2005; Franke et al., 2007). In SH-SY5Y cells, rosuvastatin has been shown to curtail the caspase-3 activity by approximately 50%, while at the same time raising the level of a pro-survival fragment, sAPP- α (Famer and Crisby, 2004). In addition, statins protect cultured cortical neurons from excitotoxicity after exposure to *N*-methyl *D*-aspartate (NMDA) (Zacco et al., 2003) and monosodium glutamate (Bosel et al., 2005). Furthermore, in a rabbit ischemic stroke model, simvastatin exhibits neuroprotective effects by inhibiting Rho associated kinase (ROCK) (Lapchak and Han, 2010) and in a mouse model for Parkinson's disease, simvastatin prevents dopaminergic neuronal loss by inhibiting the activation of Ras (Ghosh et al., 2009). On the contrary, lovastatin has been shown to enhance apoptosis and tau protein phosphorylation in neurons via regulation of the Rho family of GTPase (Meske et al., 2003; Ghosh et al., 2009). Interestingly, among all commercially available statins, simvastatin was found to be the most effective at protecting against kainate-induced excitotoxicity and memory impairment (Ramirez et al., 2011).

Statins have also been shown to protect neurons from A β -associated apoptosis by activating various anti-apoptotic pathways. For example, lovastatin enhances Wnt signaling pathway by stabilizing β -catenin through inhibition of glycogen synthase kinase 3 β (GSK-3 β) (Salins et al., 2007), thus protecting against A β -induced neurotoxicity (Meske et al., 2003).

Atorvastatin also promotes neuron survival and plasticity through catenin pathway in a rat model of stroke (Cespedes-Rubio et al., 2010). Furthermore, simvastatin and lovastatin promote cell survival by activation of phosphoinositide 3-kinase (PI3-K)/Akt pathway and mitogen activated protein kinase (MAPK) pathway (Kretz et al., 2006; Xu et al., 2008). In addition, atorvastatin increases cell survival by enhancing transforming growth factor β 1 (TGF- β 1)/Smad signaling pathway (Rodriguez-Vita et al., 2008). Although the underlying mechanisms by which statins regulate signaling molecules are not fully understood, inhibition of RhoA/Rock pathway has been shown to mediate some of the effects of statins on aforementioned signaling pathways (Rodriguez-Vita et al., 2008; Cespedes-Rubio et al., 2010).

Effects on cognitive function and synaptic plasticity

Many studies on the cognitive effects of statins have focused on the potential therapeutic benefits of statins for the treatment of stroke or traumatic brain injury (Balduini et al., 2003; Chen et al., 2003; Lu et al., 2007; Wu et al., 2008). For example, in rat models, chronic administration of atorvastatin or simvastatin following traumatic brain injury have been shown to improve rehabilitation of spatial memory (Lu et al., 2007), reduce inflammatory cytokine production (Balduini et al., 2003), and improve cerebral blood flow to the injury site (Chen et al., 2003). Furthermore, statins stimulate angiogenesis, neurogenesis, synaptogenesis in treated animals (Chen et al., 2003; Lu et al., 2007), and promote neurite outgrowth in cultured hippocampal neurons (Pooler et al., 2006). Though, it is noteworthy that several studies indicate that under some conditions (e.g., high concentrations), statins may decrease neurite outgrowth or synaptogenesis (Schulz et al., 2004; Kannan et al., 2010; Vural and Tuglu, 2011). Recently, the therapeutic potential of statins in AD has been investigated, largely prompted by the findings from epidemiological studies that show a markedly lower prevalence of AD/dementia in statin-prescribed populations (Jick et al., 2000; Wolozin et al., 2000). However, while some prospective studies showed that statin treatment improved cognitive function in normocholesterolemic patients (Simons et al., 2002) or slowed the decline in cognitive function of AD patients (Sparks et al., 2005), others show no protection of statins in preventing AD in a group of patients at risk for cardiovascular disease (Collins et al., 2002; Shepherd et al., 2002). In rare cases, use of statin has also been associated with cognitive impairment (Wagstaff et al., 2003). The possible reasons for such discrepancy have been reviewed thoroughly recently (Shepardson et al., 2010b; Shepardson et al., 2010a). Experimentally, we reported that chronic simvastatin treatment rescued hippocampal-dependent learning and memory in a transgenic mouse model of AD independent of changes in A β pathology (Li et al., 2006). Interestingly, dramatic memory improvements were also observed in non-transgenic wild-type littermate controls (Li et al., 2006), indicating a general pro-cognitive effect on brain function. A similar effect has been observed in adult rats treated with simvastatin for twenty-five days prior to testing in passive avoidance or object-in-place tasks (Douma et al., 2011).

In mammalian systems, the molecular and cellular changes mediating the induction and maintenance of long-term potentiation (LTP), a long-lasting synaptic enhancement, in the hippocampus are widely considered to be the basis for explicit memory formation and storage (McGaugh, 2000). In AD, dysfunction of cholinergic and glutamatergic synapses in the hippocampus and neocortex occurs prior to apparent neuronal degeneration (Selkoe, 2002). Thus, rescuing synaptic function could be an effective early intervention against AD. Atorvastatin has been shown to increase synaptic plasticity in the hippocampus of rats following acute *in vivo* administration of A β (Clarke et al., 2007). However, these potentially beneficial effects of statins are countered by a report that acute *in vitro* treatment of mouse hippocampal slices with mevastatin (also known as compactin; a statin not approved for human use) inhibits LTP at CA3-CA1 synapses (Kotti et al., 2006; Kotti et al., 2008). Recently, we have demonstrated that treatment of hippocampal slices for several hours with simvastatin increases the magnitude of N-methyl-D-aspartic acid receptor (NMDAR)-dependent LTP in the CA1 region in the hippocampus of adult wild-type mice (Mans et al., 2010; Mans et al., 2011). The reasons for the discrepancy between the studies may be related to the differences in the type of statins, the LTP induction protocols, and the age of the animals used (Mans et al., 2010).

The molecular mechanisms underlying the effects of statins on synaptic plasticity and cognitive function have not been fully elucidated. Likely, statins exert their pro-synaptic/pro-cognitive effects through multiple pathways (Fig. 2). We have shown that enhancement of learning and memory by chronic simvastatin treatment is associated with an increased level of activated (phosphorylated) Akt (p-Akt) in the brain of wild-type mice (Li et al., 2006). Consistent with our results, the PI3-K/p-Akt signaling pathway has been implicated in synaptic plasticity and memory consolidation (Horwood et al., 2006). Recently, we further demonstrated that a PI3-K inhibitor, LY-294002, selectively reduces LTP in simvastatin-treated slices (Mans et al., 2010), suggesting that PI3-K signaling is necessary for simvastatin-induced LTP enhancement.

Several lines of evidence indicate that statin-induced depletion of isoprenoids and subsequent inhibition of small GTPase prenylation are responsible for modulating cognitive and synaptic functions. In a mouse model of neurofibromatosis type 1 (NF1) mental retardation, caused by excessive p21 Ras activity, lovastatin normalizes p21Ras activity and reverses deficits in LTP and spatial learning (Li et al., 2005). Consistently, several other studies have shown that Ras negatively regulates NMDAR transmission. H-Ras overexpression decreases tyrosine phosphorylation of NMDAR NR2A subunit and the magnitude of LTP (Thornton et al., 2003), and inhibiting a Ras effector protein, RACK1, increases NMDAR currents in hippocampal neurons (Yaka et al., 2002). Conversely, H-Ras-deficient mice display enhanced tyrosine phosphorylation of NMDAR NR2A and NR2B subunits that occurs with an accompanying increase in NMDAR conductance and LTP magnitude (Manabe et al., 2000). These findings strongly suggest that statin-mediated Ras inhibition could account for the augmentation of NMDAR-dependent plasticity in statin-treated animals. Recently, we found that the LTP-enhancing property of simvastatin is specifically blocked by supplementation of FPP, the substrate for farnesylation (Mans et al., 2011). Our observations corroborate several reports (Manabe et al., 2000; Yaka et al., 2002;

Thornton et al., 2003; Li et al., 2005) demonstrating a negative relationship between NMDAR-dependent synaptic plasticity and the farnesylation pathway. As the brains of AD patients produce elevated amounts of FPP and GGPP (Eckert et al., 2009), it is plausible that elevated isoprenoid production may be directly detrimental to synaptic and cognitive function, and therefore statins may present a potential treatment for synaptic and cognitive disorders.

BISPHOSPHONATES

Bisphosphonates (BPs) are chemically stable analogues of natural pyrophosphate compounds that normally prevents calcification of soft tissues, and regulates bone mineralization (Fleisch et al., 1966). BPs had been available hundreds of years but it was only 40 years ago that they were discovered as effective inhibitors of bone resorption (Fleisch et al., 1969a; Fleisch et al., 1969b). Since then, numerous BPs have been synthesized and many of them are approved for treating bone diseases of excessive bone resorption (Russell, 2011). Based on their mechanisms of action, BPs can be categorized into two groups: the non-nitrogen BPs and nitrogen-containing BPs. The non-nitrogen BPs, such as etidronate and clodronate, have simple chemical structures. They act by incorporating into toxic, non-hydrolyzable ATP analogues (Rogers et al., 2011). The more potent, nitrogen-containing BPs (N-BPs), such as pamidronate, neridronate, risedronate, zoledronate, minodronate, alendronate, and ibandronate, inhibit the enzymes in the mevalonate pathway (Rogers et al., 2011). The main target of N-BPs is farnesyl pyrophosphate synthase (FPPS), downstream of HMG-CoA reductase where statins act (Fig. 1). This section will be focused on the current state of research on N-BPs (Table 2).

Mechanisms of action of N-BPs

The therapeutic application of N-BPs attributes to the properties common to all BPs: their backbone P-C-P structure and the ability to chelate calcium ions. Therefore, they are targeted rapidly to bone mineral surface *in vivo*, where they are taken up mainly by osteoclasts (bone-destroying cells). Emerging evidence, however, indicates that other endocytic cells such as monocytes and macrophages may also internalize BPs that are present transiently in the circulation (Roelofs et al., 2010). Once inside the cell, N-BPs, through yet an unknown intracellular pathway, bind FPPS and inhibit its activity, leading to a depletion of FPP and GGPP. Consequently, the prenylation process is impaired, causing loss of membrane attachment of proteins such as small GTPases, loss of interactions with other proteins and downstream signaling, and/or accumulation of unprenylated proteins and therefore inappropriate activation of downstream signaling pathways. Concurrently, inhibition of FPPS also results in the accumulation of upstream metabolite IPP (Fig. 1), which leads to the formation of new metabolites such as ApppI (an ATP analog) (Rogers et al., 2011). Although N-BPs may act on additional molecular targets, these two mechanisms, inhibition of protein prenylation and accumulation of ApppI, are mainly responsible for the anti-resorptive and apoptosis-inducing effects of these agents on osteoclasts (Rogers et al., 2011). By inhibiting bone resorption and inducing osteoclast apoptosis, N-BPs reduce bone turnover, increase bone mass and improve bone mineralization.

Interestingly, the effects of N-BPs on osteoclasts are mediated primarily through inhibition of protein geranylgeranylation rather than protein farnesylation, even though N-BPs deplete both GGPP and FPP. This is supported by experiments in which supplementation of geranylgeraniol, which is readily converted to GGPP for protein geranylgeranylation, but not farnesol, which is readily converted to FPP for protein farnesylation, abolishes the effects of N-BPs on inhibiting osteoclast formation and bone resorption (Fisher et al., 1999). Additional evidence comes from studies in which specific prenylation inhibitors were used. Whereas loss of geranylgeranylated proteins in osteoclasts in the presence of a GGT inhibitor blocks bone resorption, loss of farnesylated proteins in the presence of an FT inhibitor has little effect (Coxon et al., 2000). Therefore, geranylgeranylated proteins appear to play a significantly more important role in regulating the functions of osteoclasts than farnesylated proteins.

Potential applications of N-BPs in diseases other than bone disorders

Therapeutic applications of N-BPs in other conditions are limited by their rapid targeting to bone. However, studies have shown that N-BPs possess strong anti-tumor activities *in vitro* and in some *in vivo* models of cancer through FPPS inhibition in tumor cells (Cleazardin et al., 2005). Additionally, N-BPs may have therapeutic potential for Hutchinson–Gilford progeria syndrome (HGPS), caused by mutations in nuclear lamin A protein and subsequent accumulation of prenylated mutant lamin A (Young et al., 2006; Dechat et al., 2007). Treatment of N-BPs in combination with statins was found to attenuate the aging symptoms and extends longevity in a mouse model of HGPS (Varela et al., 2008). The combined treatment effectively inhibits the prenylation process and therefore blocks abnormal membrane localization and function of mutant lamin A in the nucleus of HGPS cells (Varela et al., 2008). Furthermore, some evidence indicates that N-BPs may have positive effects on neurodegenerative disorders such as Alzheimer's disease. An *in vitro* study shows that N-BPs prevent the oxidative stress-induced inactivation of human brain muscarinic acetylcholine receptors exposed to Alzheimer's brain extract (Fawcett et al., 2002). An animal experiment demonstrates that a BBB permeable N-BP suppresses the activity of acetylcholinesterase (AChE) in the frontal cortex of rats (Cibickova et al., 2007), implicating the potential benefits of N-BPs in AD as AChE inhibitors are commonly used to treat patients with AD. The recent finding that levels of FPP and GGPP are elevated significantly in the brain of Alzheimer's patients (Eckert et al., 2009) also suggests that treatment with FPPS inhibitors to reduce downstream isoprenoids may provide therapeutic benefits. However, development of prodrugs of N-BPs or other types of FPPS inhibitors, which circumvent the tissue distribution limitations of these compounds, will be necessary for clinical use of these drugs in treating neurological disorders. Alternatively, a drug delivery vehicle can be developed to deliver and protect N-BPs until they are released in the CNS.

Much effort has been made to remove the phosphonate groups of N-BPs so that they would be accessible to other tissues. Interestingly, even subtle modifications to the phosphonate groups (such as methylation) that reduce binding to bone mineral also decrease the ability of these compounds to inhibit protein prenylation because the phosphonate groups appear to be critical for interacting with the substrate binding site of FPPS (Luckman et al., 1998; Kavanagh et al., 2006; Rondeau et al., 2006). Recently, novel non-BP inhibitors of FPPS

that bind to an allosteric site on the enzyme have been identified (Jahnke et al., 2010). These inhibitors lack the phosphonate groups of N-BPs and thus do not bind to bone mineral, offering the hope that this new class of compounds may lead to a much broader therapeutic applications of FPPS inhibitors clinically.

PRENYLTRANSFERASE INHIBITORS

As discussed above, both statins and N-BPs function by limiting the availability of prenylation substrates, FPP and GGPP. Therefore, they affect protein prenylation broadly and nonspecifically. Prenylation reactions are catalyzed by one of the three prenyltransferases: FT, GGT-I, and GGT-II. While protein targets for FT and GGT-I include CAAX-containing farnesylated proteins (Ras, nuclear lamins, and others) and geranylgeranylated proteins (Rho/Rac families and others), respectively, Rab protein family members are exclusive substrates of GGT-II, of which much less is known (Leung et al., 2006). To target the prenylation of certain proteins, selective inhibitors have been developed to curtail the activity of FT and GGT-I specifically and several of them are in clinical trials (Tsimberidou et al., 2010; Berndt et al., 2011) (Table 3).

Farnesyl transferase inhibitors (FTIs)

Initial interest in developing FTIs was prompted by the finding that approximately 30% of all human cancers harbor activating oncogenic mutations in the Ras genes (mainly H-Ras, K-Ras and N-Ras) and that farnesylation was absolutely required for the malignant transforming activity of mutated Ras GTPases (Kohl et al., 1993). The importance of farnesylation in tumorigenesis is underscored recently by tissue-specific genetic deletion of FT in mouse models, in which the mutant K-Ras-induced lung cancer is hampered and the lifespan extended (Liu et al., 2010), further validating the efforts of developing FTIs as anti-cancer therapies.

A variety of FTIs have been developed and investigated in anticancer clinical trials. These include peptide analog inhibitors that mimic and compete with substrates of FT, CAAX-containing proteins or FPP, as well as nonpeptidomimetic inhibitors or compounds that were identified from high throughput screening of large compound libraries. Several FTIs, including tipifarnib, lonafarnib, BMS-214662, and L-778,123, have been or are being evaluated in clinical trials (Table 3). FTI treatment results in the reversal of several hallmarks of cancer, including mitotic arrest at prometaphase (Crespo et al., 2001; Crespo et al., 2002), induction of apoptosis (Barrington et al., 1998; Zhang et al., 2002), inhibition of anchorage-dependent and anchorage-independent growth (Reuveni et al., 2003), reduction of invasion and angiogenesis (Cohen-Jonathan et al., 2001; Kim et al., 2010), and induction of tumor regression in animal models. Although preclinical studies indicate that FTIs are highly effective as anti-cancer agents, clinical trials with FTIs have only produced limited success (recently reviewed in (Berndt et al., 2011)). The reason for such poor clinical outcome is not completely clear but partly due to the lack of complete understanding on the mechanism of action of FTIs and the obscurity of specific farnesylated proteins critical for the development of certain cancers. Another possibility relates to the fact that K-Ras and N-Ras can be geranylgeranylated by GGT when FT is inhibited and remain fully functional (Lerner et al., 1997; Rowell et al., 1997; Whyte et al., 1997), therefore escaping the

inhibition by FTIs. This situation prompts the effort of developing GGT inhibitors (GGTIs) (discussed below).

The application of FTIs has extended beyond cancers. As with statins and N-BPs, FTIs have been shown to offer beneficial effects on Hutchinson-Gilford progeria syndrome (HGPS), in which mutant lamin A protein remains persistently farnesylated and anchored in the nuclear membrane, leading to its aberrant behavior during interphase and mitosis (Young et al., 2006; Dechat et al., 2007). Numerous studies have shown that treating HGPS cells with FTIs can block, and possibly even reverse, abnormalities caused by mutant lamin A (recently reviewed by in (Mehta et al., 2010)). Furthermore, in animal models of HGPS, treatment with an FTI (ABT-100) has also been found to attenuate several of progeroid phenotypes with respect to body weight, fat deposition and bone density, and increased life expectancy (Fong et al., 2006; Yang et al., 2006). A more recent study demonstrated that treatment with an FTI (R115777, Zarnestra) retards the onset, as well as the progression, of cardiovascular diseases in the human mutant lamin A transgenic mouse model (Capell et al., 2008). These successful findings in animal models are the driving force for the initiation of clinical trials using FTIs to ameliorate or reverse the disease process in progeria children (Kieran et al., 2007). In addition, a short-term treatment with an FTI has been shown to aid the therapy for cardiovascular disease by inhibiting neointima formation and preventing restenosis after balloon angioplasty (Work et al., 2001).

The therapeutic potential of FTIs for parasitic diseases (such as malaria) has also been explored (Eastman et al., 2006). These FTIs have been designed specifically to inhibit parasitic FT but not mammalian FT (Carrico et al., 2004; Nallan et al., 2005). In addition, some viruses (such as hepatitis D virus) depend on protein farnesylation for their virion assembly. Thus, FTIs have been tested for their antiviral activity and shown to be highly effective at clearing viremia in mice infected with hepatitis D virus (Bordier et al., 2003), suggesting their potential as an antiviral agent.

A potential neurological application of FTIs has also emerged recently. FTIs have been used to rescue synaptic and cognitive deficits caused by overactivation of farnesylated proteins. For instance, members in the Ras superfamily, many of which depends on farnesylation for their function, play important roles in neuronal plasticity and memory formation (Ye and Carew, 2010). Treatment with an FTI (BMS 191563) attenuates the activity of mutant Ras and reverses learning and memory impairment in a mouse model of NF1 (Costa et al., 2002). As discussed earlier, farnesylation of H-Ras negatively regulates synaptic plasticity (Manabe et al., 2000; Yaka et al., 2002; Thornton et al., 2003) and genetic deletion of H-Ras leads to the enhancement of hippocampal LTP (Manabe et al., 2000). Consistent with these findings, recently we have shown that an FTI (FTI-277) enhances the magnitude of LTP in the hippocampus of WT mice (Mans et al., 2011), implicating the potential of FTIs as a modulator of synaptic function even under normal non-diseased conditions. Whether FTIs have any effects on the development of Alzheimer's disease has not been investigated. However, the recent finding that the level of FPP is elevated in the brain of AD patients (Eckert et al., 2009) suggests that the abundance of farnesylated proteins could be increased, contributing to the pathogenesis of AD. Therefore, use of FTIs could potentially offer beneficial effects on AD. In addition to small GTPases, FTIs can modify the function of

other farnesylated proteins. Recently, an FTI (FTI-277) was found to attenuate alpha-synuclein-induced neurotoxicity by reducing the membrane association of farnesylated ubiquitin C-terminal hydrolase-L1 (UCH-L1) that is linked to Parkinson's disease (PD) and memory (Liu et al., 2009).

Geranylgeranyl transferase inhibitors (GGTIs)

The finding that some farnesylated proteins, such as K-Ras and N-Ras, can escape FTI-mediated inhibition and remain fully functional via geranylgeranylation by GGT underscores the importance of developing GGTIs, as well as FTIs, for cancer therapy. The benefits of simultaneous inhibition of FT and GGT against tumorigenesis were further supported by conditional knockout mouse models, in which simultaneous ablation of FT and GGT produced a far greater effect on mutant K-Ras induced lung cancer onset and progression than either deletion alone (Liu et al., 2010). Several GGTIs have been developed, and similar to FTIs, GGTIs have been shown to inhibit tumor cell growth in cultured cells and in animal models (Berndt et al., 2011). One GGTI (GGTI-2418) is currently being tested in clinical trials (O'Dwyer et al., 2010) (Table 3).

The application of GGTIs in diseases other than cancers has also been explored. GGTIs may be able to facilitate the therapy for cardiovascular disease by blocking neointima formation after balloon angioplasty and improving endothelial function (upregulation of nitric oxide synthase), through inhibition of RhoA and Rac1 (Finder et al., 1997; Zuckerbraun et al., 2005). Also, several studies have shown that GGTIs can directly act on neuronal cells and the central nervous system. In rat hippocampal neurons, a GGTI (GGTI-286) enhances neuritogenesis (Pooler et al., 2006). Similarly, GGTI-286 promotes neurite outgrowth in the culture of rat embryonic cortex explants and postnatal spinal cord explants, suggesting its potential in promoting axon regeneration in brain and spinal cord injury (Holmberg et al., 2006). Another GGTI, GGTI-298, has been shown to enhance survival, proliferation, and differentiation of cultured glial cells and promote myelin repair in a rat model of experimental autoimmune encephalomyelitis (Paintlia et al., 2008). In addition, the fact that more proteins are subjected to geranylgeranylation than farnesylation (Reid et al., 2004) suggests that GGTIs could play an even broader modulatory role than FTIs. For example, small GTPases such as Rho and Rac, which require geranylgeranylation to function properly, are implicated in regulating APP/A β metabolism (Cole et al., 2005; Ostrowski et al., 2007; Zhou et al., 2008), neuronal migration and development (Govek et al., 2011), axon extension and dendritic structure (Tolias et al., 2011), and neuronal plasticity and memory formation (Newey et al., 2005). Therefore, GGTIs may be a potential therapeutic agent for AD and other cognitive impairment disorders.

CONCLUSIONS AND PERSPECTIVES

Development of isoprenoid- and prenylation-related interventions has been spurred by the importance of growing numbers of prenylated proteins in health and disease (Perez-Sala, 2007). Although remarkable progress has been made toward the treatment of cardiovascular and bone diseases and some forms of cancers, the full therapeutic potential of isoprenoid- and prenylation-related agents remains to be achieved.

One of the challenges facing the field of prenylation is the enormous scope and complexity of prenylated proteins. Even though numerous biologically important proteins have been identified to undergo prenylation (Lane and Beese, 2006), the exact size of the prenylome is unknown (Maurer-Stroh et al., 2007). Based on the literature, several hundred proteins are subjected to prenylation (Perez-Sala, 2007; Berndt et al., 2011). The impact of prenylated proteins certainly extends beyond what has been shown experimentally. Most of the studies have focused on the prenylation of individual proteins and therefore have only touched the tip of the iceberg. Recently, methods are being developed to characterize protein prenylation globally. One approach is to label cellular proteins with detectable FPP and/or GGPP analogs (Kho et al., 2004; Chan et al., 2009; Nguyen et al., 2009; DeGraw et al., 2010; Onono et al., 2010) and another approach uses a sequence-based computer program specially designed to predict if proteins are prenylated (Maurer-Stroh et al., 2007). Application of these approaches will enable a quantitative proteome-wide analysis of the regulation of protein prenylation and its modulation by therapeutic agents. Also, as these techniques become feasible in general laboratories and clinics, prenylomic profiling of individuals can be performed to facilitate personalized medicine, decoding inter-individual differences in the response to therapeutic agents.

Finally, to develop highly selective and effective therapeutic agents, it will be crucial to identify specific prenylated proteins that play critical roles in the pathogenesis of relevant diseases. Recently generated conditional knockout mice, in which FT and GGT activities can be ablated in a tissue specific manner (Liu et al., 2010), provide an invaluable *in vivo* model system to investigate the entire spectrum of influence of prenylation inhibition. In conjunction with aforementioned approaches to characterize the prenylome, these animal models offer an unprecedented opportunity for elucidating the mechanisms of disease, identifying and validating therapeutic targets, and ultimately improving the outcomes of clinical trials.

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Fig. 1.

The mevalonate pathway. Pharmacological agents inhibit the activity of key enzymes in the mevalonate pathway (Goldstein and Brown, 1990) and elicit a series of metabolic and functional changes of downstream molecules (see text for details). PP, pyrophosphate; FPPS, farnesyl PP synthase; FT, farnesyl transferase; FTIs, farnesyl transferase inhibitors; GGT, geranylgeranyl transferase; GGTIs, geranylgeranyl transferase inhibitors.

Fig. 2.

Potential pathways by which statins exert pro-synaptic/pro-cognitive effects. Statins may counteract detrimental effects of noxious stimuli such as amyloid- β ($A\beta$) through multiple pathways (O'Dell et al., 1994; Essig et al., 1998; Lin et al., 2001; Vaughan, 2003; Pang et al., 2004; Sweatt, 2004; Magrane et al., 2005; Snyder et al., 2005; Li et al., 2006; Clarke et al., 2007; Mans et al., 2010; Mans et al., 2011). PI3K/Akt, phosphoinositide 3-kinase/protein kinase B; ERK, extracellular signal regulated kinase; CREB, cAMP response element binding protein; NMDAR, N-methyl-D-aspartic acid receptor; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; tPA, tissue-type plasminogen activator; BDNF, brain-derived neurotrophic factor; sAPP α , neurotrophic fragment produced by α -secretase cleavage of amyloid- β precursor protein.

Table 1

FDA approved statins*

Generic Name	Brand Name	Lipophilic/Hydrophilic	BBB Permeability	T _{1/2} (h)	Metabolism (CYP)	LDL-C Reduction (%) ^a	Approval Date
Lovastatin	Mevacor®	Lipophilic	High	2–3	3A4	34	8/31/1991
Pravastatin	Pravachol®	Hydrophilic	Low	1.5–2	3A4 (min.)	34	10/31/1991
Simvastatin	Zocor®	Lipophilic	High	2	3A4	41	12/23/1991
Fluvastatin	Lescol®	Lipophilic	Low	1	2C9	24	12/23/1993
Atorvastatin	Lipitor®	Lipophilic	Low	14	3A4	50	12/17/1996
Rosuvastatin	Crestor®	Hydrophilic	Low	20	2C9 (min.)	63	8/12/2003
Pitavastatin	Livalo®	Lipophilic	Low	12	2C9 (min.)	48	8/9/2009

* Modified from reference (Jones, 2003; Schachter, 2005; Shitara and Sugiyama, 2006; Yee and Wright, 2011). BBB, blood-brain barrier; CYP, cytochrome P450; LDL-C, low-density lipoprotein cholesterol.

^aThe effect was obtained in hyperlipidemic patients by a daily dose of 40 mg of all statins except for pitavastatin (4 mg) (Schachter, 2005).

Table 2

FDA approved nitrogen-containing bisphosphonates

Generic Name	Brand Name	IC ₅₀ (nM) ^a	Bone Binding Affinity (μM) ^b	Metabolism ^c	Approval Date
Pamidronate	Aredia®	200	83	None	10/31/1991
Alendronate	Forsamax®	50	61	None	9/29/1995
Risedronate	Actonel® Atelvia®	10	85	None	3/27/1998 10/8/2010
Zoledronate	Zometa® Reclast®	3	81	None	8/20/2001 4/16/2007
Ibandronate	Boniva®	20	116	None	5/16/2003

^a Analyses of FPPS inhibition were conducted with recombinant human enzyme (Dunford et al., 2001).

^b Binding affinity of N-BPs for human bone (Leu et al., 2006).

^c No enzymes capable of cleaving the P–C–P bond have been discovered. N-BPs are absorbed, stored, and excreted from the body unaltered (Cremers and Papapoulos, 2011). The biological half-life of N-BPs in the blood is very brief as they rapidly bind to bone, whereas their retention in the skeleton may last a lifetime.

Table 3

Prenyltransferase inhibitors in clinical trials*

Drug	Other Name(s)	Chemical derivative	IC ₅₀ (nM)	Phase	Trials ^a	Diseases
FTIs						
Tipifarnib	R115777 Zarnestra	Imidazole-methylquinolinone	1–8	I, II, III	80	Cancers
Lonafarnib	SCH66336 Sarasar	Tricyclic carboxamine	1–10	I, II, III	28 3 1	Cancers Progeria Hepatitis D
BMS-214662		Tetrahydro-benzodiazepine	1–8	I	6	Cancers
L-778,123		Peptidomimetic	2	I	2	Cancers
GGTI						
GGTI-2418		Peptidomimetic	9.5	I		Cancers

* Information based on references (Tsimberidou et al., 2010; Berndt et al., 2011).

^aThe number of clinical trials documented at www.ClinicalTrials.gov.