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REVIEW PAPER

Beneficial Effects of Fingolimod in Alzheimer's Disease: Molecular Mechanisms and Therapeutic Potential

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Abstract

Alzheimer's disease (AD), the most common cause of dementia remains of unclear etiology with current pharmacological therapies failing to halt disease progression. Several pathophysiological mechanisms have been implicated in AD pathogenesis including amyloid-β protein (Aβ) accumulation, tau hyperphosphorylation, neuroinflammation and alterations in bioactive lipid metabolism. Sphingolipids, such as sphingosine-1-phosphate (S1P) and intracellular ceramide/S1P balance are highly implicated in central nervous system physiology as well as in AD pathogenesis. FTY720/Fingolimod, a structural sphingosine analog and S1P receptor (S1PR) modulator that is currently used in the treatment of relapsing–remitting multiple sclerosis (RRMS) has been shown to exert beneficial effects on AD progression. Recent in vitro and in vivo evidence indicate that fingolimod may suppress Aβ secretion and deposition, inhibit apoptosis and enhance brain-derived neurotrophic factor (BDNF) production. Furthermore, it regulates neuroinflammation, protects against *N*-methyl-D-aspartate (NMDA)-excitotoxicity and modulates receptor for advanced glycation end products signaling axis that is highly implicated in AD pathogenesis. This review discusses the underlying molecular mechanisms of the emerging neuroprotective role of fingolimod in AD and its therapeutic potential, aiming to shed more light on AD pathogenesis as well as direct future treatment strategies.

Keywords Alzheimer's disease · Fingolimod · S1P · Sphingolipids · Amyloid-beta

Abbreviations

Aβ	Amyloid-β protein
AChEI	Acetylcholinesterase inhibitors
AD	Alzheimer's disease
AGEs	Advanced glycation end products
APP	Aβ precursor protein
AV	Atrioventricular
BACE1	β-Site APP cleaving enzyme-1
BAFF	B-cell activating factor
BBB	Blood–brain barrier
BCL-2	B-cell lymphoma 2
BDNF	Brain-derived neurotrophic factor
BMECs	Brain microvascular endothelial cells
CERK	Ceramide kinase
CNS	Central nervous system
COX-II	Cyclooxygenase-II CXCL10: C-X-C motif chemokine 10 protein
EAE	Autoimmune encephalomyelitis
GPCR	G-protein-coupled receptor
HBEGF	Heparin-binding EGF-like growth factor
FPRL1	Formyl peptide receptor like-1
HMGB1	High-mobility group box 1
IRF-3	Interferon regulatory factor 3
JNK-I	c-Jun N-terminal kinase-I
LIF	Leukemia-inhibitory factor
LTP	Long-term potentiation
MWM	Morris water maze
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	*N*-methyl-D-aspartate
RAGE	Receptor for advanced glycation end products
MS	Multiple sclerosis
RRMS	Relapsing–remitting multiple sclerosis
S1P	Sphingosine-1-phosphate
S1PR	S1P receptor
SphK	Sphingosine kinase
STAT3	Signal transducer and activator of transcription 3
TNF-α	Tumor necrosis factor-α
TrKB	Tropomyosin-related kinase B

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VCAM-1 Vascular cell adhesion molecule-1
VEGFD Vascular endothelial growth factor D

Introduction

Alzheimer's disease (AD), the most common cause of dementia in the aging population, is a progressive neurodegenerative disorder clinically characterized by irreversible gradual cognitive decline affecting memory, orientation, language and judgment (Alves et al. 2012). Current therapeutic agents for AD including the *N*-methyl-D-aspartate (NMDA) antagonist memantine as well as the acetylcholinesterase inhibitors (AChEI) donepezil, rivastigmine and galantamine fail to halt disease progression (Alves et al. 2012). The key histopathological hallmarks of AD include the deposition of extracellular senile plaques containing amyloid-β protein (Aβ) and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein (Sanabria-Castro et al. 2017).

A growing body of evidence indicates that there are several molecular defects associated with AD pathogenesis including an imbalance of Aβ production and clearance, dysregulated tau protein phosphorylation, oxidative stress, abnormal glutamatergic neurotransmission, neuroinflammation and alterations in bioactive sphingolipid metabolism (Ceccom et al. 2014, Sanabria-Castro et al. 2017).

Sphingolipids are essential lipid components of the plasma membrane, consisting of aliphatic amino alcohols that involve sphingosine (Pruett et al. 2008). They constitute a complex family of molecules including ceramide, dihydroceramide and sphingosine-1-phosphate (S1P), acting as bioactive signaling molecules and regulating several cellular processes including cell growth, differentiation, apoptosis and autophagy (Li et al. 2014). Ceramide is the main intermediate of sphingolipid metabolism being implicated in cell differentiation, apoptosis and senescence as well as in inflammatory responses. S1P is involved in cell proliferation, apoptosis and protective autophagy, whereas dihydroceramide has been recently demonstrated to induce both autophagy-associated cell death and protective autophagy (Cruickshanks et al. 2015).

Sphingolipids are highly enriched in the central nervous system (CNS) and may exert pivotal effects on numerous neuronal functions (Haughey 2010; Olsen and Faergeman 2017). They contribute to the dynamic formation of lipid membrane microdomains regulating the function of neuronal ion channels and receptors as well as Aβ precursor protein (APP) and Aβ protein metabolism (Olsen and Faergeman 2017). They regulate the Akt/protein kinase B axis which modulates the stress response, cellular metabolism and apoptosis (Jesko et al. 2019). The molecular cross-talk observed between sphingolipids and various transcription factors including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), activator protein-1 (AP-1) and Forkhead box O3 (FOXO) has been suggested to play a crucial role in neuronal cell death and the associated immune function observed in AD. Among other family members, experimental evidence implicates S1P metabolism and signaling in the process of neurodegeneration and AD pathogenesis described in detail in the following sections:

Implication of S1P in AD Pathogenesis

S1P is derived from the degradation of ceramide into sphingosine and fatty acid by ceramidase (Groves et al. 2013; Karaca et al. 2014). It is specifically generated upon phosphorylation of sphingosine at the primary hydroxyl group by the two isoforms of sphingosine kinase (SPHK), SPHK1 and 2 (Cannavo et al. 2017; Ceccom et al. 2014; Chakrabarti et al. 2016). These enzymes are widely distributed in mammalian tissues with SPHK2 presenting the predominant isoform in the brain (Groves et al. 2013). Upon secretion, S1P can bind with high affinity to the five S1P-receptor subtypes ($S1PR_{1–5}$) that belong to the G-protein-coupled receptor (GPCR) family and are widely expressed in both neurons and glial cells (Asle-Rousta et al. 2013; Ceccom et al. 2014). S1P can act in an autocrine way modulating the function of the cell of origin or in a paracrine way affecting neighboring cells (Mizugishi et al. 2005). Although $S1PR_{1,2,3}$ exhibit a wide tissue distribution, $S1PR_4$ is predominantly expressed in immune cells and $S1PR_5$ is mainly found in spleen and CNS, mostly on oligodendrocytes (Groves et al. 2013).

Recent studies implicate S1P metabolism and signaling in the pathogenesis of neurodegenerative diseases including AD, affecting vital cellular functions such as cell survival, apoptosis, autophagy, Aβ production and aggregation (Czubowicz et al. 2019; Jesko et al. 2019, Fig. 1). Metabolic changes occurring during early stages of neurodegeneration generally upregulate ceramide-dependent pathways that induce apoptosis and decrease the levels of anti-apoptotic S1P, implying a possible causal association. Investigation of human postmortem brain tissues of AD patients has detected an imbalance in the concentration levels between ceramide and S1P that affects Aβ aggregation as well as autophagy and mitochondrial dysregulation (Czubowicz et al. 2019). S1P opposes the pro-apoptotic effects of ceramide probably by reducing oxidative stress and regulating the expression of various pro- and anti-apoptotic members of B-cell lymphoma 2 (BCL-2) family. It has been shown to upregulate ERK and p38MAPK as well as deactivate JNK through binding to membrane GPCRs (Van Brocklyn and Williams 2012). S1P levels were found to be positively correlated with neuronal death via a calcium/calpain/CDK5-dependent mechanism and

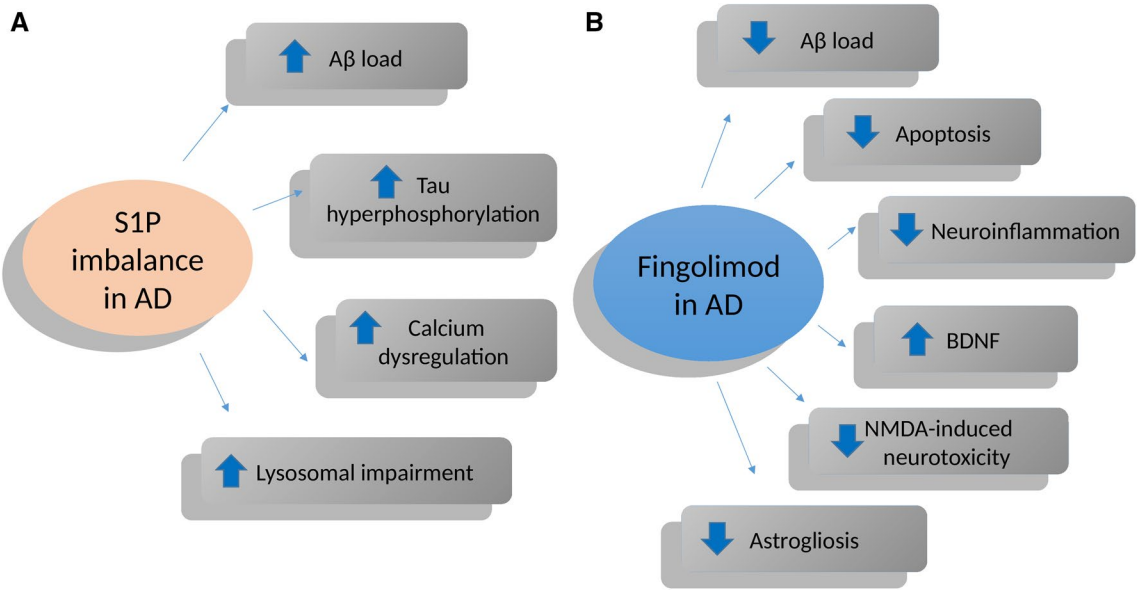

Fig. 1 Role of Sphingosine-1P imbalance in AD and potential protective role of Fingolimod. **a** Sphingosine-1-phosphate (S1P) imbalance in AD has been associated with increased Aβ load, tau hyperphosphorylation, calcium dysregulation and lysosomal impairment. **b** Studies indicate that a structural sphingosine analog and S1P receptor modulator, FTY720/Fingolimod may decrease Aβ load, apoptosis and neuroinflammation while it may increase brain-derived neurotrophic factor (BDNF). Furthermore, Fingolimod has been demonstrated to reduce *N*-methyl-D-aspartate (NMDA)-induced neurotoxicity and astrogliosis

tau hyperphosphorylation (Hagen et al. 2011). S1PRs can also activate the anti-apoptotic PI3 K/Akt axis by suppressing glycogen synthase kinase-3 β (GSK-3β) that phosphorylates tau protein as well as the protein BAD, playing a pivotal role in AD pathogenesis. On the contrary, S1P can exert opposing neurotoxic effects in some cases when its concentration is too high or related to the spatiotemporal regulation of its generation and degradation, highlighting its potential region-, cell- and time-specific effects on neurodegeneration (Czubowicz et al. 2019).

Regarding APP and Aβ metabolism, S1P has been shown to directly stimulate the β-site APP cleaving enzyme-1 (BACE1) which displays β-secretase activity, leading to Aβ production via degradation of Aβ precursor protein (APP) (Takasugi et al. 2011). In addition, S1P lyase-knock out cells display increased APP accumulation accompanied by lysosomal degradation impairment (Karaca et al. 2014). On the other hand, SPK1 overexpression has been reported to promote neuroblastoma cell survival after Aβ exposure (Gomez-Brouchet et al. 2007).

Although the implication of S1P metabolism and signaling in AD pathogenesis is still under extensive investigation, recent experimental studies suggest that pharmacological agents mimicking or regulating S1P function such as the analog, fingolimod could be beneficial against AD progression and are further discussed in the following sections:

Fingolimod Biochemistry and Activity

The S1P analog Fingolimod (2-amino-2-propane-1,3-diolhydrochloride, FTY720, trade name: Gilenya®) is the first oral disease-modifying agent approved by the US Food and Drug Administration (FDA) for relapsing remitting multiple sclerosis (RRMS). It is a synthetic fungus metabolite derived from the ascomycete *Isaria sinclairii* (Brinkmann et al. 2001) which has also been used in traditional Chinese medicine as an elixir imparting eternal youth (Strader et al. 2011). Once absorbed, fingolimod becomes an active metabolite upon phosphorylation in the cytosol by SPHK2 that forms fingolimod-P. Fingolimod-P acts as a structural S1P analog and a ligand for four of the five S1PRs ($S1PR_{1,3–5}$), exhibiting the highest affinity for $S1PR_1$ (Strader et al. 2011). Upon binding of either fingolimod-P or S1P, $S1PR_{1,3–5}$ are internalized and translocated from the cell membrane inside the cell. However, while S1P binding to $S1PR_{1,3–5}$ leads to the receptor recycling, fingolimod-P-$S1PR_{1,3–5}$ interaction results in receptor degradation indicating that fingolimod-P acts as a functional $S1PR_{1,3–5}$ agonist (Cruickshanks et al. 2015; Strader et al. 2011).

Fingolimod's immunomodulatory role in RRMS is attributed to its ability to retain auto-reactive lymphocytes in the lymph nodes and prevent them from entering into

the circulation and consequently into the CNS by $S1PR_1$ downregulation on T-cells (Hunter et al. 2016; Huwiler and Zangemeister-Wittke 2017). However, fingolimod being a lipophilic molecule can cross the blood–brain barrier (BBB) and become phosphorylated inside the brain to fingolimod-P, a charged molecule with reduced ability to cross the BBB (Asle-Rousta et al. 2014; Hunter et al. 2016).

Fingolimod has been shown to exert direct pluripotent effects in the CNS including reduction of dendritic spine loss, protection against excitotoxic neuronal death, inhibition of microglial activation, restoration of synaptic defects and reduction of astrogliosis (Hunter et al. 2016). Given the fact that these pathophysiologic processes are also observed in AD, it has been proposed that fingolimod may exhibit beneficial effects against the progression of this neurodegenerative disease.

Fingolimod Effects on APP Metabolism and Aβ Aggregation in AD

Aβ accumulation is considered as a key initiator and mediator of AD pathogenesis contributing to neuronal apoptosis, oxidative stress, neuroinflammation, formation of neurofibrillary tangles and mitochondrial dysfunction (Sun et al. 2015). Previous studies have demonstrated that fingolimod may inhibit Aβ production in neuronal cells in vitro via S1PR1- and Gi-independent pathways through the suppression of APP cleavage induced by γ-secretase (Takasugi et al. 2013). More specifically, upon fingolimod treatment, mouse primary cortical neurons displayed lower secretory levels of Abeta40 and Abeta42 in a SphK2-dependent manner while the extracellular addition of fingolimod-P did not alter Aβ secretion from neuronal cells. This suggests either a direct binding of fingolimod to APP or γ-secretase, or the fingolimod-induced redistribution and the subsequent decreased activity of γ-secretase in the plasma membrane via the β-arrestin pathway (Takasugi et al. 2013). Moreover, fingolimod may directly inhibit Sphk1 (Tonelli et al. 2010). Given the fact that the activity of Beta-secretase 1 (BACE1) which regulates Aβ formation is decreased by SphK1 inhibitors, it is possible that fingolimod may affect Aβ production via downregulation of BACE1 (Takasugi et al. 2011).

Another potential mechanism could involve the ability of fingolimod to reduce ceramide levels which can in turn enhance Aβ production (He et al. 2010; Lahiri et al. 2009). It has been demonstrated that S1P accumulation induced by the inhibition of S1P lyase, an enzyme that degrades S1P may impair lysosomal function leading to APP accumulation and Aβ production (Karaca et al. 2014). Moreover, S1P may directly promote β-secretase activity leading to increased amyloidogenesis. Thus, fingolimod acting as a S1P functional antagonist may also exert its anti-amyloidogenic properties via the same mechanism (Takasugi et al. 2011).

Collectively, intracellular phosphorylation of fingolimod may reduce Aβ production from neuronal cells in vitro, at least partially via APP cleavage suppression through a S1PR1- and Gi-independent process.

In accordance, fingolimod administration in 5xFAD transgenic mouse models of AD was shown to reduce Aβ42 and to a lesser extent Aβ40 and plaque deposition in the frontal cortex being accompanied by decreased Aβ40 and Aβ42 levels (Aytan et al. 2016).

On the contrary, fingolimod did not significantly alter Aβ plaques or soluble Aβ in presenilin 1 (PS1) and APP/PS1-transgenic AD model mice while it was shown to lower Aβ40 levels but increase Aβ42 levels in APP-transgenic mice (McManus et al. 2017; Takasugi et al. 2013). The ability of fingolimod to reduce Aβ load was correlated with SphK2 levels indicating that fingolimod-P may be responsible for Aβ reduction. However, the role of SPHK2 activity in AD human brains is controversial since some studies indicate that SPHK2 activity is increased in the frontal cortex while others have shown a reduction of SPHK2 activity in hippocampus and temporal cortex of AD brains (Couttas et al. 2014; Takasugi et al. 2011). Furthermore, the subcellular localization of SPHK2 has been shown to be dysregulated in human AD brains, as evidenced by a reduction of cytosolic SPHK2 and an increase of its nuclear levels (Dominguez et al. 2018). Therefore, the observed effects of fingolimod may depend on the animal model used, the specific brain region as well as the subcellular region under study, requiring further systematic studies to clarify its role in Aβ production and deposition.

Apart from in situ Aβ production and accumulation, it is already known that Aβ can also be transferred from the periphery into the brain through BBB via its interaction with specific receptors such as the receptor for advanced glycation end products (RAGE) (Sanabria-Castro et al. 2017). The Aβ-RAGE interaction has been shown to induce endothelial cell apoptosis, inflammation and long-term potentiation (LTP) suppression suggesting that RAGE signaling axis may play a critical role in AD pathogenesis (Sanabria-Castro et al. 2017). A recent study has demonstrated that fingolimod treatment is associated with increased levels of the two RAGE isoforms, sRAGE and esRAGE as well as with decreased levels of the two RAGE ligands, high-mobility group box 1 (HMGB1) and pentosidine in multiple sclerosis (MS) patients (Sternberg et al. 2018). Given the regulatory role of fingolimod in the RAGE axis, it is tempting to speculate that it could also mediate its effects on Aβ accumulation and AD pathogenesis based on its ability to modulate Aβ transit inside the brain.

Neuroprotective Role of Fingolimod in AD

Experimental studies indicate that fingolimod may also exert neuroprotective effects in AD by several other mechanisms (Asle-Rousta et al. 2013; Doi et al. 2013; Hemmati et al. 2013; Ruiz et al. 2014). Fingolimod has been shown to protect against Aβ-induced neuronal loss in vitro by inhibiting oligomeric Aβ-induced neurotoxicity in primary mouse cortical neuronal cell cultures as well as in open microscale cultures of primary CNS cells in microfluidic chips upon exposure to oligomeric Aβ (Doi et al. 2013; Ruiz et al. 2014). Furthermore, Aβ-induced hippocampal neuronal cell death was rescued after fingolimod treatment in Aβ-injected rat AD models (Asle-Rousta et al. 2013; Hemmati et al. 2013).

The underlying molecular mechanisms of fingolimod's neuroprotective effects against Aβ neurotoxicity have been shown to involve the inhibition of apoptosis by suppressing caspase-3 activation and reducing the Aβ-induced transcription of pro-apoptotic genes encoding caspase-3, c-Jun N-terminal kinase-I (JNK-I) and p38 (Asle-Rousta et al. 2013; Hemmati et al. 2013). Caspase-3 activation has been demonstrated to lead to APP cleavage, amyloid plaque development and neurofibrillary tangle formation (Chu et al. 2017). Current pharmacological agents against AD, memantine and AcHEI have been associated with caspase-3 inactivation (Shen et al. 2010; Yazawa et al. 2006).

JNKs have been implicated in AD pathogenesis leading to activation of pro-apoptotic signaling pathways via c-Jun phosphorylation, amyloid plaque deposition and tau hyperphosphorylation (Yarza et al. 2015).

Activation of the p38 MAPK pathway has also been linked to various pathophysiological processes of AD including synaptic dysfunction, NMDA-induced excitotoxicity and tau hyperphosphorylation (Munoz and Ammit 2010). A recent study showed that fingolimod administration may enhance pro-survival signaling by elevating *Bcl2*, *SphK1*, *SphK2* and ceramide kinase *(CerK)* gene expression in an APP-transgenic AD mouse model (Jesko et al. 2018). Therefore, fingolimod is implicated in the prevention of these pathogenic mechanisms makes it a promising neuroprotective candidate against AD progression.

It has been already well-established that BDNF exerts critical neurotrophic effects in the CNS through its contribution to memory formation, neuronal cell survival, synaptic plasticity, neuronal transmission and axonal growth (Cunha et al. 2010). BDNF is also highly implicated in AD pathology since it has been associated with attenuation of Aβ aggregation, Aβ-induced neurotoxicity and synaptic dysfunction (Jiao et al. 2016). Recent evidence indicates that fingolimod may increase Bdnf levels in mouse primary cortical neuron cells upon Aβ exposure through the interaction of Bdnf with its receptor tropomyosin-related kinase B (TrKB) and the subsequent activation of Erk1/2 signaling (Doi et al. 2013). Additionally, Bdnf production was restored in Aβ-injected AD mouse models upon fingolimod administration (Fukumoto et al. 2014). However, another study showed that fingolimod did not increase Bdnf mRNA levels in a rat AD model, highlighting the need for further studies to confirm fingolimod's effects in Bdnf production (Hemmati et al. 2013).

Apart from its interaction with S1PRs, fingolimod may also be phosphorylated by SPHK2 in the nucleus and inhibit class I histone deacetylases (HDACs), thus promoting histone acetylation and inducing altered gene expression. Fingolimod has been shown to inhibit hippocampal HDACs in vitro and in vivo and regulate the expression of many genes associated with learning and memory such as *Bdnf*, Nuclear Receptor Subfamily 4 Group A Member 2 (*Nr4a2*), a neuroprotective molecule with anti-inflammatory properties and Vascular Endothelial Growth Factor D (*VegfD*), a growth factor playing a critical role in memory formation (Hait et al. 2014; Mauceri et al. 2011; Montarolo et al. 2016). Fingolimod-induced epigenetic changes in hippocampal gene expression profile may therefore present an alternative mechanism to promote memory formation and to stimulate anti-inflammatory properties in AD.

Fingolimod has also been demonstrated to act neuroprotectively against NMDA-induced excitotoxic neuronal death via its interaction with S1PRs (Di Menna et al. 2013). Fingolimod treatment was shown to reduce glutamate levels in the brain of a mouse AD model as assessed by magnetic resonance spectroscopy, highlighting its potential protective role over AD excitotoxicity (Aytan et al. 2016; Dong et al. 2009).

In addition, fingolimod administration was shown to trigger the survival and proliferation of neuronal progenitors in the hippocampus of adult mice (Efstathopoulos et al. 2015). Given the presence of S1PRs in neural progenitor cells, fingolimod could stimulate neurogenesis via S1PR interaction and prove beneficial in AD where impaired neurogenesis has been shown to contribute to cognitive decline (Groves et al. 2013; Hollands et al. 2016).

Furthermore, elevated ceramide levels have been correlated with neuronal cell death in AD, whereas S1P may exert neuroprotective effects on Aβ-induced neurotoxicity (Cutler et al. 2004). Increased ceramide and decreased S1P levels have been associated with Aβ peptide load and p-Tau levels in AD patients (He et al. 2010). Moreover, Aβ-induced cell death has been associated with an increased ceramide/S1P ratio and SphK overexpression in vitro (Gomez-Brouchet et al. 2007). Therefore, the neuroprotective role of fingolimod may also be attributed to its regulatory role over the ceramide/S1P levels, but this requires further investigation (Asle-Rousta et al. 2013).

Fingolimod as a Critical Modulator of Neuroinflammation in AD

Increasing evidence indicates that neuroinflammation plays a crucial regulatory role in AD pathogenesis. Astrocyte and microglia activation as well as peripheral immune cell infiltration into the brain have been found to contribute to the Aβ-induced neuronal cell loss, synaptic dysfunction and oxidative stress (Sawikr et al. 2017). Aβ itself has been suggested to trigger the production of pro-inflammatory cytokines and ROS by monocytes, microglia and neutrophils as well as induce chemotaxis of monocytes (Bianca et al. 1999; Kaneider et al. 2004; Lorton 1997). Activated microglia and astrocytes have been shown to surround amyloid plaques and secrete pro-inflammatory proteins including TNF-α, IL-1β and cyclooxygenase (COX-II) (Hohsfield and Humpel 2015). On the other hand, activated microglia may exert protective roles via Aβ phagocytosis, leading to plaque clearance (Hensley 2010). Epidemiological evidence has revealed that the use of nonsteroidal anti-inflammatory drugs is associated with a reduced risk of AD development (Scarpini et al. 2003). As previously mentioned treatment with fingolimod results in the retaining of auto-reactive lymphocytes inside lymph nodes on RRMS and inhibits their penetration into the CNS via its interaction with S1PRs which are also expressed in resident glial cells in addition to neurons and peripheral immune cells. It is, therefore, suggested that fingolimod may affect AD pathogenesis via its regulation of inflammation in both the CNS and the periphery.

Additionally, fingolimod may regulate microglial activation since it can downregulate NF-κB activity and the subsequent production of the pro-inflammatory cytokines TNF-α and IL-1 produced by activated microglia and astrocytes in cell cultures (Jackson et al. 2011; Zhong et al. 2018). It was also shown to inhibit microglial activation and reduce the number of activated microglia in ischemic brain lesions in mouse models and in the 5xFAD-transgenic mouse model of AD (Aytan et al. 2016; Czech et al. 2009). Moreover, fingolimod suppressed gene transcription of *Nf-κB*, *Tnf-α* and *IL-1* in the hippocampus of Aβ-injected AD models in a SphK2-dependent manner, mediated via $S1PR_1$ signaling in the brain (Asle-Rousta et al. 2014; Hemmati et al. 2013). This is of high importance since NF-κB signaling is known to play a pivotal role in AD pathogenesis and is involved in Aβ production, oxidative stress and neuroinflammation (Collister and Albensi 2005; Snow and Albensi 2016).

Fingolimod was also demonstrated to increase the gene transcription of Interferon regulatory factor 3 (*Irf-3*), a key transcription factor that induces the switch of the pro-inflammatory M1 phenotype of microglia to the anti-inflammatory M2 state in a white matter ischemia model, possibly via induction of Stat3 signaling (Qin et al. 2017). This switch was also suggested to take place in AD rat models with neuroprotective benefits (Hemmati et al. 2013; Tarassishin et al. 2011).

Apart from microglia activation, reactive astrocytosis is also increasingly recognized as an important mechanism underlying AD pathogenesis. Fingolimod treatment has been shown to reduce astrocytosis in the hippocampal regions of a murine AD model (Aytan et al. 2016). This is in agreement with previous studies demonstrating the inhibition of pro-inflammatory cytokine production by astrocytes in vitro and the reduction of astrogliosis in an animal model of autoimmune encephalomyelitis (EAE) (Choi et al. 2011; Van Doorn et al. 2010). Furthermore, fingolimod was shown to trigger the production of neurotrophic mediators such as IL-11, leukemia-inhibitory factor (LIF) and Heparin-binding epidermal growth factor (HBEGF) from astrocytes while it reduced the astrocytic expression of TNF-induced genes, B-cell-activating factor (*BAFF*), chemokine interferon-γ inducible protein 10 (*CXCL10*) and Myxovirus (Influenza Virus) Resistance 1 (*MX1*) (Hoffmann et al. 2015). It was further shown to attenuate astrocyte-induced neuronal loss in murine EAE models by blocking the production of nitric oxide (NO) and IL-17, IL-1 and S1P-induced translocation of NF-κB in astrocytes (Colombo et al. 2014).

In AD, fingolimod was demonstrated to attenuate astrocytic activation and BBB permeability while it also increased Aβ phagocytosis by astrocytes in PS1 and APP/PS1-transgenic AD mice infected by *Bordetella pertussis* (McManus et al. 2017). This data indicate that fingolimod exerts beneficial effects in acute inflammation-induced AD cases, such as after infections, which are already known to accelerate cognitive impairment (Holmes et al. 2003).

Although infiltration of peripheral blood leucocytes is less prominent in AD brain as compared to MS, it has been demonstrated that Aβ may actually trigger immune cell migration into the CNS contributing to neurodegeneration via uncontrolled neuroinflammation (Hohsfield and Humpel 2015). The Aβ-induced chemotaxis of peripheral monocytes has been shown to be mediated through the activation of formyl peptide receptor like-1 (FPRL1), a GPCR family receptor (Cui et al. 2002). Of note, Aβ and prion proteins act as FPRL1 ligands and prion-FPRL1 interaction has been reported to activate S1P-signalling (Kaneider et al. 2003; Shen et al. 2000). Previous studies have demonstrated that fingolimod may attenuate Aβ- and APP-induced migration of human peripheral monocytes in a SPHK-dependent manner via interaction with S1PRs and S1P signalling (Kaneider et al. 2004). Therefore, fingolimod treatment may inhibit Aβ-induced peripheral monocyte migration in AD brain. However, given the fact that infiltrated leucocytes may also

exert beneficial effects on AD pathogenesis by inducing Aβ clearance, further studies are needed (Simard et al. 2006).

During the development of many neurological diseases including AD, the BBB, which is mainly composed of brain microvascular endothelial cells (BMECs) and periendothelial structures such as the basal membrane, astrocytes and pericytes, is partially disrupted, allowing peripheral leucocytes to penetrate more easily into the CNS (Kaneider et al. 2004; van Sorge and Doran 2012). BMECs have been reported to express S1PRs and SPHK2, indicating that fingolimod may impact BBB integrity by regulating their function (Nishihara et al. 2015). In MS patients, fingolimod has been demonstrated to reduce BBB leakiness via downregulation of vascular cell adhesion molecule-1 (VCAM-1) and upregulation of claudin-5 in BMECs. VCAM-1 is an adhesion molecule playing a key role in the migration of leucocytes through the BBB and claudin-5 is one of the main components of tight junctions (Nishihara et al. 2015). Interestingly, VCAM-1 has been found to be elevated in AD patients, being highly implicated in microvascular inflammation (Zenaro et al. 2017). Fingolimod was shown to affect the endothelial cells of BBB via its direct interaction with $S1P_5$ (van Doorn et al. 2012). Consequently, fingolimod may inhibit the disruption of BBB integrity observed in AD via upregulation of VCAM-1, but this requires further investigation.

Fingolimod Effects on Cognitive Function in Alzheimer's Disease: Therapeutic Potential

Fingolimod has been shown to restore cognitive decline in a white matter ischemic injury model and improve memory impairment in rats upon brain ischemia (Qin et al. 2017; Nazari et al. 2016). Importantly, its regulatory role on neuroinflammation and neuroprotection are accompanied by beneficial effects on cognitive deficits in AD animal models (Table 1). Fingolimod treatment has been shown to restore Aβ-induced learning and memory deficits in AD rat models assessed by the Morris Water Maze (MWM) test (Asle-Rousta et al. 2013; Asle-Rousta et al. 2014). It has been demonstrated to inhibit learning and memory impairment in both male and female AD rat models as assessed by the passive avoidance test with comparable effects to the approved AD drug, memantine (Hemmati et al. 2013). Additionally, fingolimod attenuated Aβ-induced associative learning and object recognition memory deficits in AD mouse models inhibiting Aβ-induced impairment of the context-dependent freezing response which is generally hippocampus-dependent, but with no effect on the tone-dependent freezing response, that is amygdala-dependent (Fukumoto et al. 2014). Consequently, the neuroprotective effects of fingolimod may have a significant clinical impact in ameliorating or maybe delaying cognitive decline in AD patients.

Given the possible neuroprotective effects of fingolimod in AD animal models, it has also been proposed to serve as a potential preventive and/or therapeutic strategy for humans. However, $S1PR_1$ are expressed in almost all cell types and chronic fingolimod treatment may induce prolonged downregulation of $S1PR_1$ leading to multiple effects on cellular functions with several adverse effects (Huwiler and Zangemeister-Wittke 2017). In particular, fingolimod administration has been associated with potentially serious cardiovascular complications such as first-dose bradycardia and atrioventricular (AV) blockade (Yoshii et al. 2017). For this reason, absolute contraindications for fingolimod in MS include recent (past 6 months) transient ischemic attack, stroke, myocardial infarction, unstable angina, class III/IV heart failure and/or decompensated heart failure requiring hospitalization as well as sick sinus syndrome without a functioning pace-maker, Mobitz type II second-degree or third-degree AV block, long QTc interval ($\geq$ 500 ms) at baseline and/or treatment with antiarrhythmic drugs of class Ia or III group (Singer 2013). Fingolimod may also increase the risk of infections such as *Varicella* virus infection as well as progressive multifocal leukoencephalopathy. Furthermore, significantly elevated liver enzymes and conditions associated with increased risk of macular edema such as uveitis may also outweigh the benefits of its use.

Importantly, since fingolimod is classified as pregnancy category C due to in vivo evidence of indicated teratogenicity, its use would not be recommended for women of childbearing potential (Singer 2013). Consequently, in considering therapeutic potential of fingolimod in AD, cautious case selection and screening is necessary before treatment initiation to maximize both its effectiveness and safety, especially for the elderly AD patients with co-existing medical problems.

Based on the above evidence, ideal AD patients that could benefit from the use of fingolimod would be young and immunosufficient without any condition predisposing to macular edema or other cardiovascular or liver disease, without concurrent treatment with immunosuppressant or antiarrhythmic drugs and effective birth control for women of childbearing potential.

Because of the potential serious side effects, another target group of cases could be selected presymptomatic subjects carrying *PS1*, *PS2* or *APP* mutations that cause autosomal dominant AD since these cases usually develop AD at a relatively early age (approximately from 30 to 50 years) and suffer from minimal comorbidities (Bateman et al. 2011). Additionally, pathogenic variants of these genes display complete penetrance, suggesting that all carriers will manifest AD (Bateman et al. 2011). Furthermore, the relatively stereotyped onset age of the disease in these cases can be

Table 1 Experimental studies demonstrating Fingolimod's effects in AD

Type of study	Cell culture/AD animal model	Fingolimod effects	References
In vitro	Primary cortical neurons	Inhibition of Aβ40 and Aβ42 secretion Suppression of APP cleavage induced by γ-secretase	Takasugi et al. (2013)
In vitro	Primary cortical neurons	Inhibition of oligomeric Aβ-induced neurotoxicity	Doi et al. (2013)
In vitro	Open microscale cultures of primary CNS cells	Inhibition of oligomeric Aβ-induced neuronal loss	Ruiz et al. (2014)
In vitro	Primary cortical neurons	Increase of Bdnf levels upon Aβ exposure	Doi et al. (2013)
In vitro	Human peripheral monocytes	Attenuation of Aβ- and APP-induced migration	Kaneider et al. (2004)
In vitro	Primary cultured microglia	Reduction of Aβ42-induced NF-κB activity Reduction of Aβ42-induced pro-inflammatory cytokine generation	Zhong et al. (2018)
In vivo	5xFAD-transgenic model mice of AD	Reduction of Aβ42, and to a lesser extent of Aβ40 and plaque deposition in the cortex Reduction of glutamate levels detected by magnetic resonance spectroscopy (MRS) Reduction of the number of activated microglia Reduction of astrocytosis in the hippocampus	Aytan et al. (2016)
In vivo	APP-transgenic AD mice	Reduction of Aβ40 levels but increase of Aβ42 levels.	Takasugi et al. (2013)
In vivo	Aβ-injected rat model of AD	Aβ-induced hippocampal neuronal cell death Inhibition of apoptosis via suppressing caspase-3 activation Restoration of Aβ-induced learning and memory deficits	Asle-Rousta et al. (2013)
In vivo	Aβ-injected rat model of AD	Reduction of TNF-α and COX-II levels Restoration of Aβ-induced learning and memory deficits	Asle-Rousta et al. (2014)
In vivo	Aβ-injected rat model of AD	Aβ-induced hippocampal neuronal cell death Reduction of the Aβ-induced transcription of pro-apoptotic genes encoding caspase-3, c-Jun N-terminal kinase-1 (JNK-1) and p38 No change of BDNF mRNA levels Suppression of gene transcription of *Nf-κB, Tnf-α* and *IL-1* in the hippocampus Increase of *Irf-3* gene transcription Inhibition of learning and memory impairment	Hemmati et al. (2013)
In vivo	APP-transgenic AD model mice	Enhancement of pro-survival signaling, by elevating B-cell lymphoma 2 *(Bcl2), SphK1, SphK2* and ceramide kinase *(CerK)* gene expression	Jesko et al. (2018)
In vivo	Aβ-injected AD model mice	Restoration of Bdnf production via the interaction of Bdnf with its receptor tropomyosin-related kinase B (TrKB) and the subsequent activation of ERK1/2 signaling Attenuation of learning deficits.	Fukumoto et al. (2014)
In vivo	PS1 and APP/PS1-transgenic AD model mice	Attenuation of astrocytic activation and BBB permeability only in AD mice infected by *Bordetella pertussis* Decrease of Aβ plaques and soluble Aβ only in AD mice infected by *Bordetella pertussis* Increase in Aβ phagocytosis by astrocytes only in AD mice infected by *Bordetella pertussis*	McManus et al. (2017)

well predicted, facilitating the design of prevention clinical trials (Cummings et al. 2012).

Given the fact that fingolimod effects have been investigated mainly in AD animal models carrying human AD-causing mutations, results from clinical trials with patients carrying these pathogenic variants are supposed to be "closer" to the in vivo evidence described above (Bateman et al. 2011). Moreover, prodromal AD cases defined as patients with no functional disability and dementia but exhibiting a hippocampal type amnestic syndrome as well as a positive biomarker of AD could also serve as a target group (Cummings et al. 2012). Finally, given the recent

evidence that younger patients may have a significantly faster disease progression, the benefits of more aggressive treatments such as fingolimod could outweigh the possible risks (Bernick et al. 2012).

In this context, the effects of fingolimod were investigated in a recent Phase II clinical trial that was carried out in patients with another neurodegenerative disorder, Amyotrophic Lateral Sclerosis, without any serious adverse events being reported. A transient heart rate slowing and total lymphocyte count reduction were observed in the fingolimod-treated group compared to placebo but liver function tests did not show any significant alterations (Mendell et al. 2017).

Finally, to overcome the ubiquitous effects of fingolimod to $S1PR_1$, additional more selective compounds able to bind to specific S1PR subtypes have been developed. Such molecules include ponesimod, cenerimod and KRP-203 which are $S1PR_1$ selective as well as ozanimod and siponimod which are $S1PR_{1+5}$ selective (Huwiler and Zangemeister-Wittke 2017). These drugs may prove very effective for autoimmune disorders as well as AD, possibly displaying greater efficacy and tolerability.

Concluding Remarks

Experimental studies investigating the importance of S1PR modulators and fingolimod in AD pathogenesis are still limited. However, emerging evidence highlights the importance of sphingolipid dysregulation and targeting of the S1PRs as a potential novel treatment option for AD progression (Clausznitzer et al. 2018).

The molecular mechanisms underlying fingolimod action in AD involve the regulation of Aβ production, accumulation and transit in the CNS, inhibition of Aβ-induced neuronal cell apoptosis as well as BDNF production, neurogenesis, and inhibition of HDACs in the nucleus. In addition, regulation of microglial activation and reactive astrocytosis, suppression of peripheral monocyte infiltration and inhibition of BBB disruption have also been associated with fingolimod's treatment. Given the complicated pathophysiological processes that characterize AD pathogenesis, including neuronal loss, Aβ deposition and neuroinflammation, these pleiotropic effects of fingolimod make it a promising novel candidate for AD treatment.

Future studies should be focused on the elucidation of the specific region-, cell- and time-specific effects and underlying molecular mechanisms of fingolimod and other S1PR modulators in AD progression to develop more selective compounds with better efficacy and fewer adverse effects.

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References

Alves, L., Correia, A. S., Miguel, R., Alegria, P., & Bugalho, P. (2012). Alzheimer's disease: A clinical practice-oriented review. *Frontiers in Neurology, 3,* 63. https://doi.org/10.3389/fneur.2012.00063.

Asle-Rousta, M., Kolahdooz, Z., Dargahi, L., Ahmadiani, A., & Nasoohi, S. (2014). Prominence of central sphingosine-1-phosphate receptor-1 in attenuating abeta-induced injury by fingolimod. *Journal of Molecular Neuroscience, 54*(4), 698–703. https://doi.org/10.1007/s12031-014-0423-3.

Asle-Rousta, M., Kolahdooz, Z., Oryan, S., Ahmadiani, A., & Dargahi, L. (2013). FTY720 (fingolimod) attenuates beta-amyloid peptide (Abeta42)-induced impairment of spatial learning and memory in rats. *Journal of Molecular Neuroscience, 50*(3), 524–532. https://doi.org/10.1007/s12031-013-9979-6.

Aytan, N., Choi, J. K., Carreras, I., Brinkmann, V., Kowall, N. W., Jenkins, B. G., et al. (2016). Fingolimod modulates multiple neuroinflammatory markers in a mouse model of Alzheimer's disease. *Scientific Reports, 6,* 24939. https://doi.org/10.1038/srep24939.

Bateman, R. J., Aisen, P. S., De Strooper, B., Fox, N. C., Lemere, C. A., Ringman, J. M., et al. (2011). Autosomal-dominant Alzheimer's disease: A review and proposal for the prevention of Alzheimer's disease. *Alzheimer's Research & Therapy, 3*(1), 1. https://doi.org/10.1186/alzrt59.

Bernick, C., Cummings, J., Raman, R., Sun, X., & Aisen, P. (2012). Age and rate of cognitive decline in Alzheimer disease: implications for clinical trials. *Archieves of Neurology, 69*(7), 901–905. https://doi.org/10.1001/archneurol.2011.3758.

Bianca, V. D., Dusi, S., Bianchini, E., Dal Pra, I., & Rossi, F. (1999). Beta-amyloid activates the O-2 forming NADPH oxidase in microglia, monocytes, and neutrophils. A possible inflammatory mechanism of neuronal damage in Alzheimer's disease. *The Journal of Biological Chemistry, 274*(22), 15493–15499. https://doi.org/10.1074/jbc.274.22.15493.

Brinkmann, V., Pinschewer, D. D., Feng, L., & Chen, S. (2001). FTY720: Altered lymphocyte traffic results in allograft protection. *Transplantation, 72*(5), 764–769.

Cannavo, A., Liccardo, D., Komici, K., Corbi, G., de Lucia, C., Femminella, G. D., et al. (2017). Sphingosine kinases and sphingosine 1-phosphate receptors: Signaling and actions in the cardiovascular system. *Frontiers in Pharmacology, 8,* 556. https://doi.org/10.3389/fphar.2017.00556.

Ceccom, J., Loukh, N., Lauwers-Cances, V., Touriol, C., Nicaise, Y., Gentil, C., et al. (2014). Reduced sphingosine kinase-1 and enhanced sphingosine 1-phosphate lyase expression demonstrate deregulated sphingosine 1-phosphate signaling in Alzheimer's disease. *Acta Neuropathologica Communications, 2,* 12. https://doi.org/10.1186/2051-5960-2-12.

Chakrabarti, S. S., Bir, A., Poddar, J., Sinha, M., Ganguly, A., & Chakrabarti, S. (2016). Ceramide and sphingosine-1-phosphate in cell death pathways: Relevance to the pathogenesis of alzheimer's disease. *Current Alzheimer Research, 13*(11), 1232–1248. https://doi.org/10.2174/1567205013666160603004239.

Choi, J. W., Gardell, S. E., Herr, D. R., Rivera, R., Lee, C. W., Noguchi, K., et al. (2011). FTY720 (fingolimod) efficacy in an animal

model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor 1 (S1P1) modulation. *Proceedings of the National academy of Sciences of the United States of America, 108*(2), 751–756. https://doi.org/10.1073/pnas.1014154108.

Chu, J., Lauretti, E., & Pratico, D. (2017). Caspase-3-dependent cleavage of Akt modulates tau phosphorylation via GSK3beta kinase: Implications for Alzheimer's disease. *Molecular Psychiatry, 22*(7), 1002–1008. https://doi.org/10.1038/mp.2016.214.

Clausznitzer, D., Pichardo-Almarza, C., Relo, A. L., van Bergeijk, J., van der Kam, E., Laplanche, L., et al. (2018). Quantitative systems pharmacology model for alzheimer disease indicates targeting sphingolipid dysregulation as potential treatment option. *CPT Pharmacometrics & Systems Pharmacology, 7*(11), 759–770. https://doi.org/10.1002/psp4.12351.

Collister, K. A., & Albensi, B. C. (2005). Potential therapeutic targets in the NF-kappaB pathway for Alzheimer's disease. *Drug News & Perspectives, 18*(10), 623–629. https://doi.org/10.1358/dnp.2005.18.10.959576.

Colombo, E., Di Dario, M., Capitolo, E., Chaabane, L., Newcombe, J., Martino, G., et al. (2014). Fingolimod may support neuroprotection via blockade of astrocyte nitric oxide. *Annals of Neurology, 76*(3), 325–337. https://doi.org/10.1002/ana.24217.

Couttas, T. A., Kain, N., Daniels, B., Lim, X. Y., Shepherd, C., Kril, J., et al. (2014). Loss of the neuroprotective factor Sphingosine 1-phosphate early in Alzheimer's disease pathogenesis. *Acta Neuropathologica Communications, 2,* 9. https://doi.org/10.1186/2051-5960-2-9.

Cruickshanks, N., Roberts, J. L., Bareford, M. D., Tavallai, M., Poklepovic, A., Booth, L., et al. (2015). Differential regulation of autophagy and cell viability by ceramide species. *Cancer Biology & Therapy, 16,* 733–742. https://doi.org/10.1080/15384047.2015.1026509.

Cui, Y., Le, Y., Yazawa, H., Gong, W., & Wang, J. M. (2002). Potential role of the formyl peptide receptor-like 1 (FPRL1) in inflammatory aspects of Alzheimer's disease. *Journal of Leukocyte Biology, 72*(4), 628–635.

Cummings, J., Gould, H., & Zhong, K. (2012). Advances in designs for Alzheimer's disease clinical trials. *American Journal of Neurodegenerative Disease, 1*(3), 205–216.

Cunha, C., Brambilla, R., & Thomas, K. L. (2010). A simple role for BDNF in learning and memory? *Frontiers in Molecular Neuroscience, 3,* 1. https://doi.org/10.3389/neuro.02.001.2010.

Cutler, R. G., Kelly, J., Storie, K., Pedersen, W. A., Tammara, A., Hatanpaa, K., et al. (2004). Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. *Proceedings of the National academy of Sciences of the United States of America, 101*(7), 2070–2075. https://doi.org/10.1073/pnas.0305799101.

Czech, B., Pfeilschifter, W., Mazaheri-Omrani, N., Strobel, M. A., Kahles, T., Neumann-Haefelin, T., et al. (2009). The immunomodulatory sphingosine 1-phosphate analog FTY720 reduces lesion size and improves neurological outcome in a mouse model of cerebral ischemia. *Biochemical and Biophysical Research Communications, 389*(2), 251–256. https://doi.org/10.1016/j.bbrc.2009.08.142.

Czubowicz, K., Jesko, H., Wencel, P., Lukiw, W. J., & Strosznajder, R. P. (2019). The role of ceramide and sphingosine-1-phosphate in Alzheimer's disease and other neurodegenerative disorders. *Molecular Neurobiology*. https://doi.org/10.1007/s12035-018-1448-3.

Di Menna, L., Molinaro, G., Di Nuzzo, L., Riozzi, B., Zappulla, C., Pozzilli, C., et al. (2013). Fingolimod protects cultured cortical neurons against excitotoxic death. *Pharmacological Research, 67*(1), 1–9. https://doi.org/10.1016/j.phrs.2012.10.004.

Doi, Y., Takeuchi, H., Horiuchi, H., Hanyu, T., Kawanokuchi, J., Jin, S., et al. (2013). Fingolimod phosphate attenuates oligomeric amyloid beta-induced neurotoxicity via increased brain-derived neurotrophic factor expression in neurons. *PLoS ONE, 8*(4), e61988. https://doi.org/10.1371/journal.pone.0061988.

Dominguez, G., Maddelein, M. L., Pucelle, M., Nicaise, Y., Maurage, C. A., Duyckaerts, C., et al. (2018). Neuronal sphingosine kinase 2 subcellular localization is altered in Alzheimer's disease brain. *Acta Neuropathologica Communications, 6*(1), 25. https://doi.org/10.1186/s40478-018-0527-z.

Dong, X. X., Wang, Y., & Qin, Z. H. (2009). Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacologica Sinica, 30*(4), 379–387. https://doi.org/10.1038/aps.2009.24.

Efstathopoulos, P., Kourgiantaki, A., Karali, K., Sidiropoulou, K., Margioris, A. N., Gravanis, A., et al. (2015). Fingolimod induces neurogenesis in adult mouse hippocampus and improves contextual fear memory. *Translational Psychiatry, 5,* e685. https://doi.org/10.1038/tp.2015.179.

Fukumoto, K., Mizoguchi, H., Takeuchi, H., Horiuchi, H., Kawanokuchi, J., Jin, S., et al. (2014). Fingolimod increases brain-derived neurotrophic factor levels and ameliorates amyloid beta-induced memory impairment. *Behavioural Brain Research, 268,* 88–93. https://doi.org/10.1016/j.bbr.2014.03.046.

Gomez-Brouchet, A., Pchejetski, D., Brizuela, L., Garcia, V., Altie, M. F., Maddelein, M. L., et al. (2007). Critical role for sphingosine kinase-1 in regulating survival of neuroblastoma cells exposed to amyloid-beta peptide. *Molecular Pharmacology, 72*(2), 341–349. https://doi.org/10.1124/mol.106.033738.

Groves, A., Kihara, Y., & Chun, J. (2013). Fingolimod: direct CNS effects of sphingosine 1-phosphate (S1P) receptor modulation and implications in multiple sclerosis therapy. *Journal of the Neurological Sciences, 328*(1–2), 9–18. https://doi.org/10.1016/j.jns.2013.02.011.

Hagen, N., Hans, M., Hartmann, D., Swandulla, D., & van Echten-Deckert, G. (2011). Sphingosine-1-phosphate links glycosphingolipid metabolism to neurodegeneration via a calpain-mediated mechanism. *Cell Death and Differantiation, 18*(8), 1356–1365. https://doi.org/10.1038/cdd.2011.7.

Hait, N. C., Wise, L. E., Allegood, J. C., O'Brien, M., Avni, D., Reeves, T. M., et al. (2014). Active, phosphorylated fingolimod inhibits histone deacetylases and facilitates fear extinction memory. *Nature Neuroscience, 17*(7), 971–980. https://doi.org/10.1038/nn.3728.

Haughey, N. J. (2010). Sphingolipids in neurodegeneration. *Neuromolecular Medicine, 12*(4), 301–305. https://doi.org/10.1007/s12017-010-8135-5.

He, X., Huang, Y., Li, B., Gong, C. X., & Schuchman, E. H. (2010). Deregulation of sphingolipid metabolism in Alzheimer's disease. *Neurobiology of Aging, 31*(3), 398–408. https://doi.org/10.1016/j.neurobiolaging.2008.05.010.

Hemmati, F., Dargahi, L., Nasoohi, S., Omidbakhsh, R., Mohamed, Z., Chik, Z., et al. (2013). Neurorestorative effect of FTY720 in a rat model of Alzheimer's disease: Comparison with memantine. *Behavioural Brain Research, 252,* 415–421. https://doi.org/10.1016/j.bbr.2013.06.016.

Hensley, K. (2010). Neuroinflammation in Alzheimer's disease: Mechanisms, pathologic consequences, and potential for therapeutic manipulation. *Journal of Alzheimers Disease: JAD, 21*(1), 1–14. https://doi.org/10.3233/JAD-2010-1414.

Hoffmann, F. S., Hofereiter, J., Rubsamen, H., Melms, J., Schwarz, S., Faber, H., et al. (2015). Fingolimod induces neuroprotective factors in human astrocytes. *Journal of Neuroinflammation, 12,* 184. https://doi.org/10.1186/s12974-015-0393-6.

Hohsfield, L. A., & Humpel, C. (2015). Migration of blood cells to beta-amyloid plaques in Alzheimer's disease. *Experimental Gerontology, 65,* 8–15. https://doi.org/10.1016/j.exger.2015.03.002.

Hollands, C., Bartolotti, N., & Lazarov, O. (2016). Alzheimer's disease and hippocampal adult neurogenesis. Exploring shared mechanisms. *Frontiers in Neuroscience, 10,* 178. https://doi.org/10.3389/fnins.2016.00178.

Holmes, C., El-Okl, M., Williams, A. L., Cunningham, C., Wilcockson, D., & Perry, V. H. (2003). Systemic infection, interleukin 1beta, and cognitive decline in Alzheimer's disease. *Journal of Neurology, Neurosurgery and Psychiatry, 74*(6), 788–789. https://doi.org/10.1136/jnnp.74.6.788.

Hunter, S. F., Bowen, J. D., & Reder, A. T. (2016). The direct effects of Fingolimod in the central nervous system: Implications for relapsing multiple Sclerosis. *CNS Drugs, 30*(2), 135–147. https://doi.org/10.1007/s40263-015-0297-0.

Huwiler, A., & Zangemeister-Wittke, U. (2017). The sphingosine 1-phosphate receptor modulator fingolimod as a therapeutic agent: Recent findings and new perspectives. *Pharmacology & Therapeutics, 185,* 34–49. https://doi.org/10.1016/j.pharmthera.2017.11.001.

Jackson, S. J., Giovannoni, G., & Baker, D. (2011). Fingolimod modulates microglial activation to augment markers of remyelination. *Journal of Neuroinflammation, 8,* 76. https://doi.org/10.1186/1742-2094-8-76.

Jesko, H., Wencel, P. L., Lukiw, W. J., & Strosznajder, R. P. (2018). Modulatory effects of Fingolimod (FTY720) on the expression of sphingolipid metabolism-related genes in an animal model of Alzheimer's disease. *Molecular Neurobiology, 56*(1), 174–185. https://doi.org/10.1007/s12035-018-1040-x.

Jesko, H., Stepien, A., Lukiw, W. J., & Strosznajder, R. P. (2019). The cross-talk between Sphingolipids and insulin-like growth factor signaling: Significance for aging and neurodegeneration. *Molecular Neurobiology, 56*(5), 3501–3521. https://doi.org/10.1007/s12035-018-1286-3.

Jiao, S. S., Shen, L. L., Zhu, C., Bu, X. L., Liu, Y. H., Liu, C. H., et al. (2016). Brain-derived neurotrophic factor protects against tau-related neurodegeneration of Alzheimer's disease. *Translational Psychiatry, 6*(10), e907. https://doi.org/10.1038/tp.2016.186.

Kaneider, N. C., Kaser, A., Dunzendorfer, S., Tilg, H., & Wiedermann, C. J. (2003). Sphingosine kinase-dependent migration of immature dendritic cells in response to neurotoxic prion protein fragment. *Journal of Virology, 77*(9), 5535–5539. https://doi.org/10.1128/JVI.77.9.5535-5539.2003.

Kaneider, N. C., Lindner, J., Feistritzer, C., Sturn, D. H., Mosheimer, B. A., Djanani, A. M., et al. (2004). The immune modulator FTY720 targets sphingosine-kinase-dependent migration of human monocytes in response to amyloid beta-protein and its precursor. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 18*(11), 1309–1311. https://doi.org/10.1096/fj.03-1050fje.

Karaca, I., Tamboli, I. Y., Glebov, K., Richter, J., Fell, L. H., Grimm, M. O., et al. (2014). Deficiency of sphingosine-1-phosphate lyase impairs lysosomal metabolism of the amyloid precursor protein. *Journal of Biological Chemistry, 289*(24), 16761–16772. https://doi.org/10.1074/jbc.M113.535500.

Lahiri, S., Park, H., Laviad, E. L., Lu, X., Bittman, R., & Futerman, A. H. (2009). Ceramide synthesis is modulated by the sphingosine analog FTY720 via a mixture of uncompetitive and noncompetitive inhibition in an Acyl-CoA chain length-dependent manner. *Journal of Biological Chemistry, 284*(24), 16090–16098. https://doi.org/10.1074/jbc.M807438200.

Li, Y., Li, S., Qin, X., Hou, W., Dong, H., Yao, L., et al. (2014). The pleiotropic roles of sphingolipid signaling in autophagy. *Cell Death and Disease, 22,* e1245. https://doi.org/10.1038/cddis.2014.215.

Lorton, D. (1997). beta-Amyloid-induced IL-1 beta release from an activated human monocyte cell line is calcium- and G-protein-dependent. *Mechanisms of Ageing and Development, 94*(1–3), 199–211.

Mauceri, D., Freitag, H. E., Oliveira, A. M., Bengtson, C. P., & Bading, H. (2011). Nuclear calcium-VEGFD signaling controls maintenance of dendrite arborization necessary for memory formation. *Neuron, 71*(1), 117–130. https://doi.org/10.1016/j.neuron.2011.04.022.

McManus, R. M., Finucane, O. M., Wilk, M. M., Mills, K. H. G., & Lynch, M. A. (2017). FTY720 Attenuates Infection-Induced Enhancement of Aβ Accumulation in APP/PS1 Mice by Modulating Astrocytic Activation. *Journal of Neuroimmune Pharmacology: The Official Journal of the Society on NeuroImmune Pharmacology, 12*(4), 670–681. https://doi.org/10.1007/s11481-017-9753-6.

Mendell, J., Al-Zaidy, S. A., Shell, R., et al. (2017). AVXS-101 phase 1 gene therapy clinical trial in SMA type 1: Event free survival and achievement of developmental milestones [abstract]. *Neurology, 88*(16 suppl), CT.003.

Mizugishi, K., Yamashita, T., Olivera, A., Miller, G. F., Spiegel, S., & Proia, R. L. (2005). Essential role for sphingosine kinases in neural and vascular development. *Molecular and Cellular Biology, 25*(24), 11113–11121. https://doi.org/10.1128/MCB.25.24.11113-11121.2005.

Montarolo, F., Perga, S., Martire, S., Navone, D. N., Marchet, A., Leotta, D., et al. (2016). Altered NR4A subfamily gene expression level in peripheral blood of Parkinson's and Alzheimer's disease patients. *Neurotoxicity Research, 30*(3), 338–344. https://doi.org/10.1007/s12640-016-9626-4.

Munoz, L., & Ammit, A. J. (2010). Targeting p38 MAPK pathway for the treatment of Alzheimer's disease. *Neuropharmacology, 58*(3), 561–568. https://doi.org/10.1016/j.neuropharm.2009.11.010.

Nazari, M., Keshavarz, S., Rafati, A., Namavar, M. R., & Haghani, M. (2016). Fingolimod (FTY720) improves hippocampal synaptic plasticity and memory deficit in rats following focal cerebral ischemia. *Brain Research Bulletin, 124,* 95–102. https://doi.org/10.1016/j.brainresbull.2016.04.004.

Nishihara, H., Shimizu, F., Sano, Y., Takeshita, Y., Maeda, T., Abe, M., et al. (2015). Fingolimod prevents blood-brain barrier disruption induced by the sera from patients with multiple sclerosis. *PLoS ONE, 10*(3), e0121488. https://doi.org/10.1371/journal.pone.0121488.

Olsen, A. S. B., & Faergeman, N. J. (2017). Sphingolipids: Membrane microdomains in brain development, function and neurological diseases. *Open Biology,*. https://doi.org/10.1098/rsob.170069.

Pruett, S. T., Bushnev, A., Hagedorn, K., Adiga, M., Haynes, C. A., Sullards, M. C., et al. (2008). Biodiversity of sphingoid bases ("sphingosines") and related amino alcohols. *Journal of Lipid Research, 49*(8), 1621–1639. https://doi.org/10.1194/jlr.R800012-JLR200.

Qin, C., Fan, W. H., Liu, Q., Shang, K., Murugan, M., Wu, L. J., et al. (2017). Fingolimod protects against ischemic white matter damage by modulating microglia toward M2 polarization via STAT3 pathway. *Stroke, 48*(12), 3336–3346. https://doi.org/10.1161/STROKEAHA.117.018505.

Ruiz, A., Joshi, P., Mastrangelo, R., Francolini, M., Verderio, C., & Matteoli, M. (2014). Testing Abeta toxicity on primary CNS cultures using drug-screening microfluidic chips. *Lab on a Chip, 14*(15), 2860–2866. https://doi.org/10.1039/c4lc00174e.

Sanabria-Castro, A., Alvarado-Echeverria, I., & Monge-Bonilla, C. (2017). Molecular pathogenesis of Alzheimer's disease: An update. *Annals of Neurosciences, 24*(1), 46–54. https://doi.org/10.1159/000464422.

Sawikr, Y., Yarla, N. S., Peluso, I., Kamal, M. A., Aliev, G., & Bishayee, A. (2017). Neuroinflammation in Alzheimer's disease: The preventive and therapeutic potential of polyphenolic

nutraceuticals. *Advances in Protein Chemistry and Structural Biology, 108,* 33–57. https://doi.org/10.1016/bs.apcsb.2017.02.001.

Scarpini, E., Scheltens, P., & Feldman, H. (2003). Treatment of Alzheimer's disease: Current status and new perspectives. *The Lancet. Neurology, 2*(9), 539–547. https://doi.org/10.1016/S1474-4422(03)00502-7.

Shen, H., Kihara, T., Hongo, H., Wu, X., Kem, W. R., Shimohama, S., et al. (2010). Neuroprotection by donepezil against glutamate excitotoxicity involves stimulation of alpha7 nicotinic receptors and internalization of NMDA receptors. *British Journal of Pharmacology, 161*(1), 127–139. https://doi.org/10.1111/j.1476-5381.2010.00894.x.

Shen, W., Proost, P., Li, B., Gong, W., Le, Y., Sargeant, R., et al. (2000). Activation of the chemotactic peptide receptor FPRL1 in monocytes phosphorylates the chemokine receptor CCR73 and attenuates cell responses to selected chemokines. *Biochemical and Biophysical Research Communication, 272*(1), 276–283. https://doi.org/10.1006/bbrc.2000.2770.

Simard, A. R., Soulet, D., Gowing, G., Julien, J. P., & Rivest, S. (2006). Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron, 49*(4), 489–502. https://doi.org/10.1016/j.neuron.2006.01.022.

Singer, B. A. (2013). Initiating oral fingolimod treatment in patients with multiple sclerosis. *Therapeutic Advances in Neurological Disorders, 6*(4), 269–275. https://doi.org/10.1177/1756285613491520.

Snow, W. M., & Albensi, B. C. (2016). Neuronal gene targets of NF-kappaB and their dysregulation in Alzheimer's disease. *Frontiers in Molecular Neuroscience, 9,* 118. https://doi.org/10.3389/fnmol.2016.00118.

Sternberg, Z., Kolb, C., Chadha, K., Nir, A., Nir, R., George, R., et al. (2018). Fingolimod anti-inflammatory and neuroprotective effects modulation of RAGE axis in multiple sclerosis patients. *Neuropharmacology, 130,* 71–76. https://doi.org/10.1016/j.neuropharm.2017.11.047.

Strader, C. R., Pearce, C. J., & Oberlies, N. H. (2011). Fingolimod (FTY720): A recently approved multiple sclerosis drug based on a fungal secondary metabolite. *Journal of Natural Products, 74*(4), 900–907. https://doi.org/10.1021/np2000528.

Sun, X., Chen, W. D., & Wang, Y. D. (2015). beta-Amyloid: The key peptide in the pathogenesis of Alzheimer's disease. *Frontiers in Pharmacology, 6,* 221. https://doi.org/10.3389/fphar.2015.00221.

Takasugi, N., Sasaki, T., Suzuki, K., Osawa, S., Isshiki, H., Hori, Y., et al. (2011). BACE1 activity is modulated by cell-associated sphingosine-1-phosphate. *The Journal of Neuroscience: The Official Journal of the Society of Neuroscience, 31*(18), 6850–6857. https://doi.org/10.1523/JNEUROSCI.6467-10.2011.

Takasugi, N., Sasaki, T., Ebinuma, I., Osawa, S., Isshiki, H., Takeo, K., et al. (2013). FTY720/fingolimod, a sphingosine analogue, reduces amyloid-beta production in neurons. *PLoS ONE, 8*(5), e64050. https://doi.org/10.1371/journal.pone.0064050.

Tarassishin, L., Suh, H. S., & Lee, S. C. (2011). Interferon regulatory factor 3 plays an anti-inflammatory role in microglia by activating the PI3 K/Akt pathway. *Journal of Neuroinflammation, 8,* 187. https://doi.org/10.1186/1742-2094-8-187.

Tonelli, F., Lim, K. G., Loveridge, C., Long, J., Pitson, S. M., Tigyi, G., et al. (2010). FTY720 and (S)-FTY720 vinylphosphonate inhibit sphingosine kinase 1 and promote its proteasomal degradation in human pulmonary artery smooth muscle, breast cancer and androgen-independent prostate cancer cells. *Cell Signaling, 22*(10), 1536–1542. https://doi.org/10.1016/j.cellsig.2010.05.022.

Van Brocklyn, J. R., & Williams, J. B. (2012). The control of the balance between ceramide and sphingosine-1-phosphate by sphingosine kinase: Oxidative stress and the seesaw of cell survival and death. *Comparative Biochemistry and Physiology: Part B, Biochemistry & Molecular Biology, 163*(1), 26–36. https://doi.org/10.1016/j.cbpb.2012.05.006.

Van Doorn, R., Van Horssen, J., Verzijl, D., Witte, M., Ronken, E., Van Het Hof, B., et al. (2010). Sphingosine 1-phosphate receptor 1 and 3 are upregulated in multiple sclerosis lesions. *Glia, 58*(12), 1465–1476. https://doi.org/10.1002/glia.21021.

van Doorn, R., Lopes Pinheiro, M. A., Kooij, G., Lakeman, K., van der Hof, B., van der Pol, S. M., et al. (2012). Sphingosine 1-phosphate receptor 5 mediates the immune quiescence of the human brain endothelial barrier. *Journal of Neuroinflammation, 9,* 133. https://doi.org/10.1186/1742-2094-9-133.

van Sorge, N. M., & Doran, K. S. (2012). Defense at the border: The blood-brain barrier versus bacterial foreigners. *Future Microbiology, 7*(3), 383–394. https://doi.org/10.2217/fmb.12.1.

Yarza, R., Vela, S., Solas, M., & Ramirez, M. J. (2015). c-Jun N-terminal Kinase (JNK) signaling as a therapeutic target for Alzheimer's disease. *Frontiers in pharmacology, 6,* 321. https://doi.org/10.3389/fphar.2015.00321.

Yazawa, K., Kihara, T., Shen, H., Shimmyo, Y., Niidome, T., & Sugimoto, H. (2006). Distinct mechanisms underlie distinct polyphenol-induced neuroprotection. *FEBS Letters, 580*(28–29), 6623–6628. https://doi.org/10.1016/j.febslet.2006.11.011.

Yoshii, F., Moriya, Y., Ohnuki, T., Ryo, M., & Takahashi, W. (2017). Neurological safety of fingolimod: An updated review. *Clinical & Experimental Neuroimmunology, 8*(3), 233–243. https://doi.org/10.1111/cen3.12397.

Zenaro, E., Piacentino, G., & Constantin, G. (2017). The blood-brain barrier in Alzheimer's disease. *Neurobiology of Disease, 107,* 41–56. https://doi.org/10.1016/j.nbd.2016.07.007.

Zhong, L., Jiang, X., Zhu, Z., Qin, H., Dinkins, M. B., Kong, J. N., et al. (2018). Lipid transporter Spns2 promotes microglia pro-inflammatory activation in response to amyloid-beta peptide. *Glia, 67*(3), 498–511. https://doi.org/10.1002/glia.23558.