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SPECIAL SECTION: EMERGING VOICES IN GPCR BIOLOGY—MINIREVIEW

Enhancing remyelination in multiple sclerosis via M1 muscarinic acetylcholine receptor

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ABSTRACT

Multiple sclerosis (MS) is growing in prevalence; yet, treatments that can reverse the progression of the disease are still needed. One strategy that has shown promise for reversing MS is remyelination by inhibiting the M1 receptor, a member of the muscarinic acetylcholine receptor (mAChR) family. Antagonizing the M1 mAChR is believed to be the mechanism by which clemastine, a developing drug that has been observed to enhance myelination in animal studies and phase II clinical trials, elicits its myelination-promoting effects. Recent studies have indicated that blocking M1 mAChR may promote oligodendrocyte differentiation via the extracellular signal-regulated kinase pathway, modulating Ca^{2+} concentration oscillations, and cross-talking with N-methyl-d-aspartate and Notch-1 receptors. However, clemastine has recently been found to accelerate disability in patients with MS, discouraging further progress in its clinical trials. Nevertheless, the underlying mechanisms following M1 mAChR antagonism by clemastine may still be targeted using alternative antimuscarinic drugs. This review consolidates recent advancements in our understanding of the mechanisms by which antagonizing M1 mAChR promotes remyelination and summarizes alternative antimuscarinic drugs that could be leveraged to treat MS in the future.

Significance Statement: Current treatments for multiple sclerosis are limited to disease management, and there is a need for restorative treatments that can reverse progressive forms of the disease. This review aims to summarize the potential mechanisms by which antagonizing the M1 muscarinic acetylcholine receptor could promote remyelination and elaborate on a collection of promising antimuscarinic drugs, consolidating the knowledge needed to target these mechanisms and develop therapeutics that could reverse the progress of demyelinating diseases like multiple sclerosis.

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease that affected 2.8 million people worldwide in 2020 and continues to rise in prevalence (Walton et al, 2020). MS is characterized by myelin degradation in the central nervous system associated with inflammation mediated by activated macrophages and microglia (Frischer et al., 2009). The deterioration of myelin sheaths and surrounding inflammation contributes to axonal degeneration (Frischer et al, 2009), giving rise to the characteristic white matter demyelinated lesions (Frischer et al, 2009) and gray matter atrophy of MS (Calabrese et al, 2015). Ultimately, patients with MS experience symptoms such as blindness, impaired motor function, autonomic dysfunction, and cognitive impairment that can progress to become permanent disabilities (Tafti et al, 2024).

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The clinical course of MS is organized into three phases: presymptomatic, relapsing-remitting, and secondary progressive. Patients start in the presymptomatic phase, which they stay in for an unknown duration of time. Neurological symptoms are not apparent in the presymptomatic phase, but inflammation and demyelination are visible with magnetic resonance imaging ('t Hart et al, 2021). Afterward, about 80% of patients with MS enter the relapsing-remitting MS (RRMS) phase, where neurological symptoms appear in relapsing episodes with periods of remission or recovery in between ('t Hart et al, 2021; Tafti et al, 2024). Although it is possible for patients with RRMS to recover completely from symptoms during periods of remission, residual symptoms can remain and lead to long-term disability (Tafti et al, 2024). About 60% of patients with RRMS progress to the secondary progressive phase within 5–20 years, where cycles of relapse-remission are replaced with a gradual and progressive worsening of symptoms ('t Hart et al, 2021). About 15% of patients with MS skip RRMS altogether and directly enter the progressive phase, which is known as primary progressive MS (Meca-Lallana et al, 2021). The remaining 5% of patients with MS have progressive-relapsing MS, characterized by the gradual worsening of neurological symptoms combined with relapse-remission episodes (Tafti et al, 2024).

Although these categories of MS help to organize what is clinically exhibited, some scientists have recently expressed a need for a new framework (Kuhlmann et al, 2023). How we define MS has major implications for decisions in drug development and regulation. For instance, most current US Food and Drug Administration–approved therapies focus on treating RRMS because it is the most common form of MS (Amin and Hersh, 2023). These therapies focus on modulating the immune system to reduce inflammation and shorten periods of relapse (Melchor et al, 2019). Hence, many of these therapies are limited to disease management and ineffective at treating the less common forms of MS such as secondary progressive and primary progressive (Gacem and Nait-Oumesmar, 2021). This shortcoming is argued to be a result of the current dichotomous clinical framework of MS progression (Kuhlmann et al, 2023). Instead, MS could be defined as a continuous spectrum of interplaying biological mechanisms, guiding the development of mechanism-focused therapeutics that are inclusive to more patients with MS, and helping to meet the need for treatments that stop and reverse MS progression (Kuhlmann et al, 2023). Currently, the focus is adjusting to include the development of treatments for progressive forms of MS where limited options are available (Amin and Hersh, 2023).

2. Challenges of augmenting oligodendrocytes and remyelination

One mechanism that has the potential to not only manage but reverse MS progression is remyelination (Neumann et al, 2019). Remyelination is a compensatory process that aims to repair damage from an initial insult to oligodendrocytes (OLs), the myelin-producing cells that protect neuronal axons (Neumann et al, 2019). In MS, this insult is due to autoimmune attacks that target myelin sheaths and cause them to detach from axons and disintegrate (Kuhn et al, 2019; Neumann et al, 2019). This triggers remyelination to be carried out by OL progenitor cells (OPCs) and/or surviving OLs (Neumann et al, 2019). In adulthood, pools of OPCs generated during embryonic development and early childhood are located throughout the brain and spinal cord (Yeung et al, 2014; Tsai et al, 2016; Kuhn et al, 2019). When an axon is demyelinated, chemoattractants are released to attract OPCs to the lesion (Zhao and Jacob, 2023). OPCs then proliferate and differentiate at the remyelination site to create more OPCs and new OLs, respectively (Neumann et al, 2019). Newly generated OLs produce myelin and wrap around exposed axons to help restore myelin structure (Neumann et al, 2019).

OPC proliferation and differentiation are highly regulated processes that are influenced by complex signaling pathways, growth factors, nearby cells, and epigenetic modifications (Tiane et al, 2019). Despite what we know, enhancing remyelination by targeting OPCs yields several challenges. For instance, one question is whether myelin made by newly differentiated OLs fully restores axon function. Myelin sheaths formed by newly generated OLs are thinner and shorter than original sheaths established before adulthood (Franklin and Ffrench-Constant, 2017; Kuhn et al, 2019). This may be problematic because the thickness and length of myelin sheaths are important for action potential conduction (Seidl, 2014).

Another challenge is the uncertain extent that OPC differentiation and new OLs play in facilitating remyelination in humans. Previous studies have shown that white matter myelin is remodeled when humans and animals learn (Sampaio-Baptista and Johansen-Berg, 2017). Animal studies have demonstrated an increased density of OLs in remyelinated regions (Prayoonwiwat and Rodriguez, 1993; Blakemore and Keirstead, 1999) and regions active during motor learning (Xiao et al, 2016), suggesting that newly generated OLs are responsible for remyelination and myelin remodeling. In contrast to animal models, the rate of new OL generation in adult human white matter is very low (0.32% a year) and likely cannot fully account for myelin remodeling (Yeung et al, 2014). Furthermore, a study using C14 dating on postmortem brains from patients with MS found decreased OL generation rates in remyelinated regions, suggesting that the remyelination is performed by old OLs that have survived autoimmune attacks in MS, rather than newly generated OLs as observed in animals (Yeung et al, 2019).

From these findings, some may deduce that enhancing OPC differentiation may not be the best strategy to promote remyelination in humans. However, it may be possible to overcome these challenges. As mentioned, one study found that new OLs may not be responsible for remyelination in humans. However, the same study also found that select patients with highly aggressive MS had an increased rate of new OL generation (Yeung et al, 2019). This rare phenomenon highlights that the human brain is capable of increasing new OL generation perhaps to combat MS, demonstrating the opportunity to augment OPC differentiation to promote remyelination (Yeung et al, 2019). Furthermore, modeling of C14 data from gray matter OLs revealed that OL turnover rates in the gray matter do not plateau until about 40 years of age when it subsides to 2.5%, which is notably longer and more proliferative compared with white matter (Yeung et al, 2014). Although white matter has been the focus of most MS research, gray matter damage is also implicated and may be linked to neurological symptoms (Calabrese et al, 2015). Taken together, targeting remyelination via OPC differentiation could still be relevant for treating gray matter–related pathology in patients with MS. Hence, understanding molecular factors that regulate OPC differentiation is critical for developing remyelination MS therapeutics (Franklin and Ffrench-Constant, 2017).

3. M1 receptor

One family of receptors with a role in modulating remyelination is the muscarinic acetylcholine receptors (mAChRs). The mAChR family contains 5 subtypes: M1, M2, M3, M4, and M5 mAChRs. All M receptors are G protein–coupled receptors (GPCRs) that can be activated by the key neurotransmitter acetylcholine (Kudlak and Tadi, 2023). In the brain, the most abundant muscarinic subtypes are M1, M2, and M4 mAChRs (Levey, 1993). M3 and M5 mAChR

subtypes are also present, but to a lesser extent (Levey, 1993; Reever et al, 1997). M1 mAChR is primarily located in the cerebral cortex, hippocampus, and striatum, but it is also found in the thalamus, brainstem, and cerebellum at lower levels (Levey, 1993; Kudlak and Tadi, 2023). In OPCs, the most abundant subtypes expressed are M1, M3, and M4 mAChRs (De Angelis et al, 2012). After OPCs differentiate into mature OLs, the expression of these subtypes reduces such that all M1−M5 mAChR subtypes are expressed at low levels, suggesting their involvement in mediating differentiation (De Angelis et al, 2012).

The activation of mAChRs has been observed to inhibit OPC differentiation (De Angelis et al, 2012). More recently, researchers have been directing their attention to studying the M1 mAChR, primarily due to the promise that clemastine, a developing remyelination drug, has shown. In rodent studies, clemastine has been shown to induce an array of positive effects, including improving memory disrupted by neuropathic pain (Zhu et al, 2024), reducing OPC senescence and amyloid-beta deposition in Alzheimer's disease model mice (Xie et al, 2021), and decreasing motor symptoms in MS model mice by improving OPC differentiation and myelination (Motawi et al, 2023; Ibrahim et al, 2024). As noted below (Table 1), clemastine is a general antimuscarinic and first-generation antihistamine (Lavrador et al, 2023). Hence, it is uncertain which muscarinic receptor subtype(s) it targets, or how histamine receptors are involved in the mechanism(s) by which it mediates its effects on OPCs. Furthermore, the possibility that clemastine utilizes a mechanism that depends on multiple of these receptors should also be kept in mind. However, by genetically knocking down specific mAChR subtypes and administering clemastine to mice, researchers have been able to demonstrate that clemastine promoted remyelination through a mechanism that involves antagonizing the M1 mAChR (Mei et al, 2016). Our understanding of the molecular mechanisms clemastine may use via M1 mAChR to enhance OPC differentiation and remyelination has grown substantially in recent years. Multiple mechanisms have been proposed as outlined in the next section of this review, demonstrating the complexity and multifaceted action of clemastine. By understanding these pathways, it could inform the development of more targeted remyelination therapies.

4. Mechanisms of M1 receptor in enhancing OL function

4.1. Notch-1 pathway

One pathway associated with mAChRs that may be utilized to mediate remyelination is the Notch signaling pathway. The Notch pathway is a highly conserved signaling system in animals that is implicated in a wealth of cellular processes such as cellular differentiation (Zhou et al, 2022). Notch-1, one of the four notch receptors, is expressed on OPCs and inhibits their differentiation when it is canonically activated by its ligand, Jagged-1 (John et al, 2002; Li et al, 2022; Zhou et al, 2022). Jagged-1 is a highly expressed ligand protein found on the cell surface of active astrocytes, but it is not expressed on astrocytes in remyelinated regions, revealing a potential link between Jagged-1 and inhibited remyelination (Li et al, 2022). Upon binding Jagged-1, Notch-1 undergoes a conformational change and a series of cleavages. Cleavage by γ-secretase releases the Notch intracellular domain (NICD), which translocates into the nucleus and works in a complex with recombination signal binding protein for immunoglobulin kappa J region to upregulate expression of mammalian hairy and Enhancer-of-split homologs 1 and 5 and inhibit OPC differentiation (Wang et al, 1998; Yang et al, 2018; Li et al, 2022; Fig. 1).

A recent study found that when clemastine was given to MS model rats, it reduced canonical Notch-1 signaling by reducing the expression of Jagged1 in the brain (Ibrahim et al, 2024). Furthermore, clemastine increased the expression of F3/Contactin-1 (Ibrahim et al, 2024), another ligand of Notch-1 that activates its noncanonical pathway. In contrast to the canonical Notch1 pathway, activation of noncanonical F3/Notch signaling has been found to promote OPC maturation and myelination (Hu et al, 2003; Li et al, 2022). When activated by F3/Contactin, the NICD forms a complex with recombination signal binding protein for immunoglobulin kappa J region and Deltex-1, which upregulate the expression of myelin-associated glycoprotein and promote differentiation (Hu et al, 2003; Li et al, 2022). Previously, De Angelis et al (2012) had observed that mAChR activation using muscarine increased Notch-1 expression in OPCs, indicating that mAChRs may crosstalk with the Notch signaling pathway (De Angelis et al, 2012). Taken together, it is possible that clemastine utilizes M1 mAChR

Table 1
Examples of muscarinic receptor related drugs for developing cure for MS

Drug	Drug Classification	Approval Status	Current Clinical Trials for MS	Major Outcomes of Completed Trials
Clemastine	First generation antihistamine with anticholinergic characteristics (Minigh, 2008; Turner et al, 1997)	Approved; generally used for allergic reactions (Turner et al, 1997)	Currently on-going Phase III trials (NCT05338450) and Phase I/II trials (NCT05131828, NCT06065670, and NCT05359653)[a]	Phase II clinical trial (NCT02040298) found that clemastine reduced the VEP P100[b] latency without severe side effects
Quetiapine	Atypical antipsychotics that blocks various receptor types including muscarinic receptors (Maan et al, 2023)	Approved to treat schizophrenia/ acute manic episodes and be used as adjunctive drugs for major depressive disorder (Maan et al, 2023)	No known trials currently known to be studying the drug effects on patients with MS	Phase I/II clinical trial (NCT02087631) found that the administration of quetiapine at the lowest tolerable doses led to adverse reactions (sedation and paraparesis)
Benztropine	Non-selective muscarinic antagonist (Ahuja et al, 2024)	Approved as adjunctive drugs for all types of parkinsonism; can be used to manage neuroleptic-induced extrapyramidal events (Ahuja et al, 2024)	No known trials explicitly studying or studied the drug effects on patients with MS	N/A
PIPE359	Selective M1 mAChR antagonist (Schrader et al, 2020)	Not approved yet	No known trials explicitly studying or studied for the drug effects on patients with MS	N/A
PIPE-307	Selective M1 mAChR antagonist	Not approved yet	Currently on-going phase II trials (NCT06083753)	Phase I clinical trials (NCT04725175 and NCT04941781) were completed and advanced to Phase II clinical trials

N/A, not applicable.
[a]Uncertainty in the progression of the clinical trials, since another trial (NCT03109288) reported severe side effects of the drug.
[b]VEP P100 indicates the mean latency of visual evoked potentials in 100 recordings, with a high value representing neural dysfunctions in eyes.

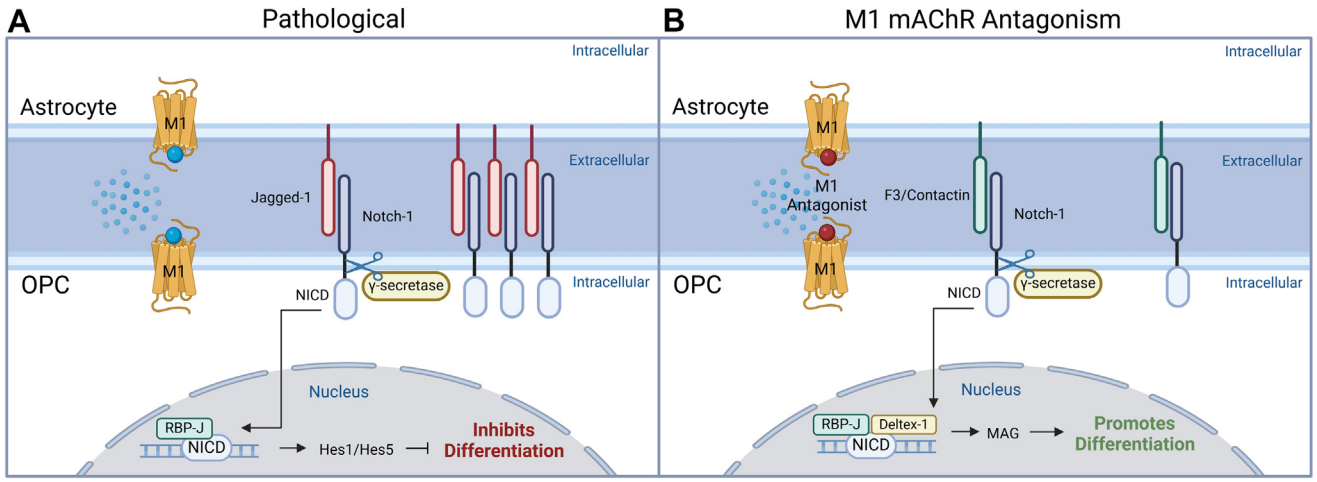

Fig. 1. Proposed mechanism by which M1 mAChR cross-talks with Notch-1 to modulate OL differentiation. (A) Jagged-1 expressed on activated astrocytes bind Notch-1 present on OPCs, triggering γ-secretase-mediated cleavage that releases NICD to complex with RBP-J. The NICD/RBP-J complex induces the expression of Hes1/Hes5 and inhibits OPC differentiation. (B) Antagonism of M1 mAChR shifts Notch-1 signaling from canonical Jagged-1 signaling to noncanonical F3/Contactin-mediated signaling that promotes OPC differentiation through the recruitment of the NICD/RBP-J/Deltex-1 complex. The specific mechanism(s) by which M1 mAChRs cross-talk with Notch-1 signaling remain unclear. The activated M1 mAChR, bound to an agonist, is depicted as a receptor associated with a blue sphere, whereas the receptor antagonist is represented by a red-brown sphere. Created using Biorender.com. Hes1/Hes5, mammalian hairy and enhancer-of-split homologs 1 and 5; RBP-J, recombination signal binding protein for immunoglobulin kappa J region.

antagonism to promote OPC differentiation by cross-talking with Notch-1 receptors, reducing their overall expression as well as shifting Jagged-1 and F3/Contactin expression of nearby cells to favor the noncanonical differentiation-promoting signaling. However, the pathways by which mAChRs may communicate with Notch-1 receptors are not understood. Furthermore, considering De Angelis et al (2012) had used muscarine, a general muscarinic agonist that is not selective for a particular mAChR subtype (Eglen and Watson, 1996), it is unclear whether this cross-talk with Notch-1 is mediated specifically by the M1 mAChR subtype. It would be interesting to investigate these gaps to distinguish which of the mAChR subtypes influences Notch-1 signaling, and whether this is driving clemastine's remyelinating effects.

Given that Jagged-1 and F3/Contactin both trigger γ-secretase-mediated NICD release when bound to Notch-1, it is interesting to consider how 2 ligands can utilize the same Notch-1 receptor to elicit differing downstream effects on OPCS. Intriguing animal research has shown that Delta-like ligand (Dll) 1 and Dll4, other ligands of Notch-1 relevant during embryonic development, facilitate contrasting responses by activating Notch-1 with different dynamics (Nandagopal et al, 2018). It was found that Dll1 activates Notch-1 in pulses to promote myogenesis, whereas Dll4 activates Notch-1 in a sustained fashion to inhibit myogenesis (Nandagopal et al, 2018). Although this study did not investigate Notch-1 on OPCs specifically, it does give insight into a possible mechanism by which Jagged-1 and F3/Contactin generate distinct effects via Notch-1. That is, it is worth considering whether Jagged-1 and F3/Contactin activate Notch-1 with differing dynamics as observed with Dll1 and Dll4 by Nandagopal et al (2018). Future research comparing how Jagged-1 and F3/Contactin activates Notch-1 differently in OPCs would be valuable for understanding how we can target Notch-1 to favor pathways that enhance OPC differentiation.

4.2. Modulating NMDA receptor expression

Clemastine and M1 mAChR may extend the duration that NMDA receptors are expressed on OPCs to elongate the period OPCs are able to differentiate. One of the cues that influences OPC differentiation is nearby neuronal activity (Foster et al, 2019; Pantazou et al, 2021), which OPCs are able to sense by expressing voltage-gated ion channels such as NMDA receptors (Spitzer et al, 2019). Over their lifespan, OPC populations acquire and lose various voltage-gated ion channels, which are believed to play a role in controlling the differentiation and proliferation of OPCs (Spitzer et al, 2019). In mice OPCs, NMDA receptors are expressed and most abundant when OPCs start differentiating and produce myelination at the highest rate (Spitzer et al, 2019). Recently, another study has found that clemastine was able to extend the duration that NMDA receptors are expressed in mice (Kamen et al, 2024). Thus, it is possible that clemastine uses M1 mAChR to modulate NMDA receptor expression to extend the period that OPCs are sensitive to external cues that promote their differentiation. However, it is unknown whether the mechanism of clemastine to influence NMDA receptor expression in OPCs specifically involves the M1 mAChR. The latest studies that investigated the connection between M1 mAChR and NMDA receptors were published over two decades ago (Calabresi et al, 1998; Marino et al, 1998). To our knowledge, there have been no studies that have investigated how M1 mAChR and NMDA receptors interact in OPCs, which is of note for future research.

4.3. Extracellular signal-regulated kinases 1/2 pathway

Another way by which clemastine could use M1 mAChR to promote myelination is through the activation of extracellular signal-regulated kinase (ERK) 1/2 in OPCs. ERK1 and ERK2 are ubiquitous mitogen-activated protein kinases, and their phosphorylation/activation regulates processes including differentiation and myelination (Gaesser and Fyffe-Maricich, 2016). When clemastine is given to rats with spinal cord injury, a condition where myelin integrity is disrupted, it was found that clemastine promotes OPC differentiation by promoting the activation of ERK1/2 (Tong et al, 2022). Additionally, when ERK1/2 signaling is inhibited via U0126, these effects are disrupted (Tong et al, 2022). Hence, the mechanism clemastine utilizes seems to depend on ERK1/2 activation. However, it is not certain whether clemastine specifically utilizes the M1 mAChR subtype to modulate ERK1/2. Although there is evidence that mAChRs (Rosenblum et al, 2000) and specifically M1 mAChR subtype (Berkeley et al, 2001) can influence ERK1/2 activation in neurons, there is a lack of research investigating the connection between M1 mAChR and ERK1/2 in

OPCs. Other areas of future research could include analyzing the conflicting findings about the impact of ERK1/2 activation on OPC differentiation and myelination (Gaesser and Fyffe-Maricich, 2016). Some studies show that ERK1/2 activation seems to promote OPC differentiation and remyelination (Tong et al, 2022; Wang et al, 2022), whereas others show that inhibiting ERK1/2 does this instead (Suo et al, 2019). This potentially indicates that the downstream signaling of ERK1/2 differs depending on the system, highlighting a need for more detailed future analysis. It is speculated that activated ERK1/2 directly enters the nucleus to activate myelin transcription factors or communicates with the Akt or Fyn pathways to drive differentiation and myelination (Gonsalvez et al, 2016). Other mitogen-activated protein kinases such as p38-γ, which has recently been found to be abundant in human MS lesions and delay differentiation of mice OPCs (Marziali et al, 2024), could also be a promising area of future study.

4.4. Preventing inhibitory Ca^{2+} oscillations

The M1 mAChR may mediate OPC differentiation by controlling Ca^{2+} concentration oscillations in OPCs. Ca^{2+} concentration can be altered upon activation of a GPCR receptor such as M1 mAChR. A series of secondary messengers are activated, depending on the type of G-protein with which the receptor is coupled. M1, M3, and M5 mAChRs are stimulatory receptors and coupled to G_q, whereas M2 and M4 are inhibitory and coupled to the G_i protein (Hulme et al, 1990; Kudlak and Tadi, 2023). G-proteins are comprised of alpha, gamma, and beta subunits. An activated G_q-coupled GPCR undergoes a conformational change to bind G_q and initiate the swapping of guanosine diphosphate for guanosine triphosphate on its alpha subunit (Sánchez-Fernández et al, 2014). The activated alpha subunit—guanosine triphosphate dissociates from the beta-gamma subunit and subsequently activates phospholipase C-beta (Berstein et al, 1992). Phospholipase C-beta catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (van der Westhuizen et al, 2020). IP3 then binds IP3 receptors located on the endoplasmic reticulum, which signals for release of Ca^{2+}, resulting in increased cytosolic Ca^{2+} concentrations (van der Westhuizen et al, 2020; Fig. 2).

To restore Ca^{2+} concentrations in the endoplasmic reticulum (ER), the cell uses a process known as store-operated calcium entry (SOCE) to pump Ca^{2+} from the extracellular space into the cell. When ER Ca^{2+} levels are low, ER-resident stromal interaction molecules sense low Ca^{2+} levels and move to regions on the ER that are adjacent to the plasma membrane (Prakriya and Lewis, 2015). Stromal interaction molecule proteins form a complex with Orai channels on the plasma membrane, allowing extracellular Ca^{2+} to flow into the cell (Prakriya and Lewis, 2015). Finally, sarcoendoplasmic reticulum calcium ATPase pumps Ca^{2+} from the intracellular space into the ER to restore Ca^{2+} stores (Prakriya and Lewis, 2015). Repeated activation of GPCRs continuously stimulates ER Ca^{2+} release and SOCE, causing oscillations of Ca^{2+} concentration in the cell (Prakriya and Lewis, 2015). The frequency of these oscillations regulates cellular processes (Smedler and Uhlén, 2014).

A recent study has found that M1 mAChR activation can cause Ca^{2+} oscillations that affect OPC differentiation (Seidman et al, 2022). The study applied various agonists of different $G_{\alpha q}$-coupled GPCRs that are expressed by human OPCs. It was found that using oxotremorine methiodide to activate M1 and M3 mAChRs could generate a prolonged Ca^{2+} oscillation response in many cells with an average frequency of 10 mHz. When the 10 mHz Ca^{2+} oscillation frequency was recreated using optogenetically induced SOCE, OPC differentiation was blocked (Seidman et al, 2022). Because oxotremorine methiodide is not a M1 mAChR-specific agonist, we cannot completely rule out the possibility that this is solely a M3 mAChR-mediated mechanism. Indeed, although the literature about the role of M3 mAChR on OPCs is not as extensive as M1 mAChR, there is evidence from human and mice OPCs that indicates M3 activity impairs OPC differentiation (Ross Welliver et al, 2018). It would be interesting to investigate whether M1 and/or M3 mAChR is responsible for facilitating the Ca^{2+} oscillation that inhibits OPC differentiation, perhaps by using selective antagonists or by genetically knocking down M1 or M3 mAChR. Additionally, the study by Seidman et al (2022) did not investigate clemastine specifically. Hence, future research could aim to confirm whether the application of clemastine prevents these oscillations in Ca^{2+} concentration to elucidate whether these oscillations are involved in its mechanism.

Another point of future research could be to investigate whether this pathway is utilized by metabotropic glutamate receptor 5 (mGluR5), another G_q-coupled GPCR that has shown to be a

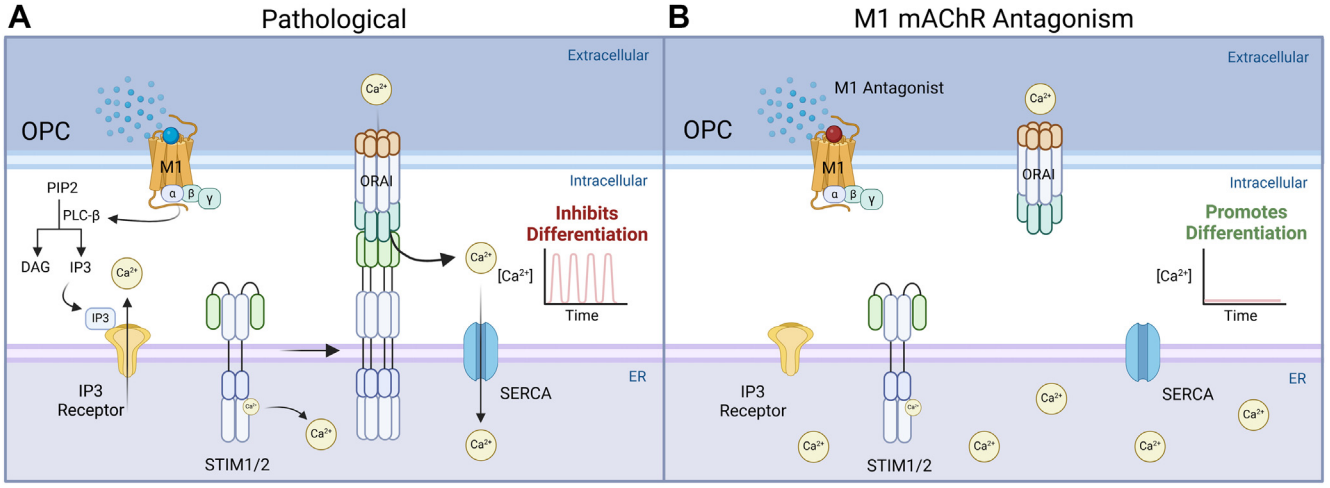

Fig. 2. Proposed mechanism by which M1 mChR affects $[Ca^{2+}]$ oscillation frequencies to mediate OL differentiation. (A) M1 mAChR activity allows for repeated release of ER Ca^{2+} via IP3 receptors, triggering STIM1/2 proteins to complex with calcium release-activated channels made up of Orai proteins, and permitting extracellular Ca^{2+} to enter the intracellular space and then be pumped into the ER via SERCA Ca^{2+} pumps. Repeated M1 mAChR activation results in oscillations of $[Ca^{2+}]$ at a frequency that inhibits OPC differentiation. (B) M1 mAChR antagonism promotes OPC differentiation by preventing inhibitory $[Ca^{2+}]$ oscillations. The activated M1 mAChR, bound to an agonist, is depicted as a receptor associated with a blue sphere, while the receptor antagonist is represented by a red-brown sphere. Created using Biorender.com. DAG, diacylglycerol; PIP2, phosphoatidylinositol-4,5-bisphopshate; PLC-β, phospholipase C-beta; SERCA, SarcoEndoplasmic reticulum calcium ATPase; STIM1/2, stromal interaction molecules 1 and 2.

promising target in the development of Alzheimer's disease therapeutics (Abd-Elrahman and Ferguson, 2022). Intriguingly, when the mGluR5 agonist 2-Chloro-5-hydroxyphenylglycine sodium salt was applied to the human OPCs, it also created the same 10 mHz Ca^{2+} oscillation frequency as seen with M1/M3 mAChR activation via oxotremorine methiodide. This frequency was distinct from the other $G_{\alpha q}$-coupled receptor ligands, suggesting the possibility that M1/M3 mAChR and mGluR5 may utilize a common downstream signaling pathway (Seidman et al, 2022). Moreover, previous research has also shown that mGluR5 and M1 mAChR may both act to modify Alzheimer's disease in a sex-specific manner (Abd-Elrahman et al, 2020, 2022, 2024), which is especially interesting as both Alzheimer's disease and MS are disproportionately more common in females than in males (Rajan et al, 2021; Gustavsson et al, 2023; Hittle et al, 2023). These parallels between mGluR5 and M1 mAChR could suggest a common sex-selective pathological mechanism relevant to both neurodegenerative diseases that warrant further study.

5. Alternatives to clemastine: other M1 drugs

In line with the promising effects clemastine had demonstrated in animal studies, clemastine yielded positive results in initial clinical trials (Table 1). The results of the phase II ReBUILD trial (NCT02040298) studied the clinical effects of clemastine and served as the earliest study to demonstrate the effectiveness of remyelinating therapies on patients with MS (Green et al, 2017). It examined the visual evoked potentials P100 as a parameter to evaluate if the remyelination could be achieved. This study found that clemastine could significantly decrease the visual evoked potential P100 latency without critical adverse reactions (Green et al, 2017). After the completion of ReBUILD trial, a phase III trial was initiated to study the long-term effect of clemastine (NCT05338450), and other phase I/II clinical trials also began to determine its remyelinating efficacy and safety (eg, NCT06065670 and NCT05359653).

Although numerous clinical studies are currently in progress to study the clinical potential of clemastine, a recent preprint article from the researchers of phase I/II clinical trial (NCT03109288) reported severe adverse effects of clemastine (Kocot et al, 2024). The clinical trial was stopped when 3 of 9 patients with MS exhibited accelerated progression of MS-caused disability (Kocot et al, 2024). They speculated that this adverse effect was elicited by clemastine amplifying the stimulation of the purinergic P2RX7-dependent cell death of myeloid cells and OLs (Kocot et al, 2024). It is not yet known how this disadvantageous characteristic of clemastine will affect the progress of other clinical trials. Perhaps lowering the dose of clemastine via combination therapy or utilizing patient screening may help avoid these adverse side effects.

Although this report on the adverse effects of clemastine could discourage its use as a remyelination therapy, alternative nonselective muscarinic antagonists can be considered. Apart from clemastine, other nonselective muscarinic antagonists can also stimulate OPC differentiation and remyelination (Melchor et al, 2019). These include quetiapine and benztropine (Melchor et al, 2019), which were determined via high-throughput assays that screened a library of candidate agents for their capability in remyelination (Deshmukh et al, 2013; Mei et al, 2014).

5.1. Nonselective muscarinic antagonists for remyelination

Quetiapine is an atypical psychiatric drug with antimuscarinic properties (Table 1). Preclinical data revealed that quetiapine may provide favorable outcomes via immune and nonimmune mechanisms (Zhornitsky et al, 2013). The in vitro study found that the quetiapine treatment led to an increase in the number of both neuroprogenitor cells and mature OLs in neurosphere cultures, via the activation of ERK1/2 (Xiao et al, 2007). Quetiapine also has immunomodulatory properties (Zhornitsky et al, 2013), which are beneficial to suppressing neuroinflammatory symptoms in MS. However, like clemastine, quetiapine was also found to cause adverse reactions in patients with MS (Metz et al, 2020). In a phase IIa trial, which studied the safety profile of quetiapine on patients with RRMS and progressive MS, it was found that 2 of 4 patients developed sedation when they received increased doses from the first subtherapeutic dose (Metz et al, 2020). One patient also had an adverse reaction (paraparesis) upon the increase in the dose (Metz et al, 2020). Metz et al (2020) recommended that further in vivo studies that can verify the efficacy of quetiapine at lower doses would be needed to pursue future clinical studies with the drug.

Another nonselective muscarinic antagonist, benztropine, was also suggested to enhance OL maturation in addition to OPC differentiation (Bible, 2012). Although preclinical data involving experimental autoimmune encephalitis indicated that benztropine can ameliorate the disease severity (Deshmukh et al, 2013), to our knowledge, no clinical trials have studied its efficacy or safety profile on patients with MS. Thus, filling the lack of knowledge in the clinical efficacy of benztropine on patients with MS may be beneficial in laying the foundation for a curative MS therapy.

5.2. M1-selective antagonists for remyelination therapy

The interest in M1 mAChR-specific effects branched from the finding that modulating M1 mAChR signaling alone could largely influence OPC differentiation (Mei et al, 2016). The pharmaceutical company Contineum Therapeutics synthetically developed M1 mAChR-selective drugs, PIPE-359 and PIPE-307, to reduce the chances of drug side effects that may arise from the blockage of different muscarinic receptor subtypes (Schrader et al, 2020). In vivo studies have shown that PIPE-359 could significantly reduce the clinical severity of myelin OL glycoprotein-induced experimental autoimmune encephalitis (Schrader et al, 2020). To our knowledge, this finding served as the first evidence that M1 mAChR-specific antagonists could elicit remyelination.

Although the study with the PIPE-359 drug did not advance into clinical trials, the phase I clinical trial was conducted with another M1 mAChR-selective antagonist PIPE-307 (NCT04725175) to learn the pharmacokinetic and tolerability profiles of the drug in healthy participants. After, the trials further progressed with a phase I trial (NCT04941781) to evaluate the binding of M1 mAChRs after one oral dose of PIPE-307 and a phase II trial (NCT06083753) to investigate the therapeutic effects of PIPE-307 in patients with MS.

6. Conclusion

Despite the recent clinical failures that demonstrated the adverse effects of clemastine, modulating M1 mAChR still appears to provide favorable outcomes in treating MS. Here, we have speculated potential mechanisms to explain how M1 mAChR inhibition may promote OPC differentiation by altering Notch-1 signaling, ERK1/2 activation and Ca^{2+} oscillations, and NMDA expression pattern to enhance remyelination. Targeting M1 mAChRs and exploring alternative drug options could be promising strategies for developing curative approaches to MS.

Abbreviations

Dll, delta-like ligand; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; GPCR, G protein—coupled receptor; IP3, inositol trisphosphate; mAChR, muscarinic acetylcholine

receptor; mGluR5, metabotropic glutamate receptor 5; MS, multiple sclerosis; NICD, Notch1 intracellular domain; OL, oligodendrocyte; OPC, oligodendrocyte progenitor cell; PIP2, phosphoatidylinositol-4,5-bisphosphate; RRMS, relapsing-remitting multiple sclerosis; SOCE, store operated calcium entry.

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Conflict of interest

The authors hold no affiliations, memberships, funding, or financial holdings to declare.

Data availability

This article contains no datasets generated or analyzed during the current study.

Authorship contributions

Wrote or contributed to the writing of the manuscript: Chen, Park, Abd-Elrahman.

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