# Potential of Antimicrobials in Multiple Sclerosis Treatment

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Multiple sclerosis (MS) is a chronic, autoimmune, demyelinating disease of the central nervous system (CNS). Microbes, including bacteria and certain viruses, particularly Epstein–Barr virus (EBV), have been linked to the pathogenesis of MS. While there is currently no cure for MS, antibiotics and antivirals have been studied as potential treatment options due to their immunomodulatory ability that results in the regulation of the immune process.

multiple sclerosis antimicrobials antibiotics treatment autoimmune disease animal models antivirals experimental autoimmune encephalomyelitis microbiota

## 1. Beta-Lactam Antibiotics

Beta-lactam antibiotics are widely used in the management and treatment of bacterial infections. This class includes penicillin (PCN), cephalosporins, carbapenems, monobactams, and beta-lactamase inhibitors. They bind to penicillin-binding proteins, which, in turn, interrupt the terminal transpeptidation process and cause loss of viability and lysis of the bacterial cell. In addition to their antimicrobial effects, beta-lactams have also been shown to modulate the immune response by several mechanisms [1]. One of the mechanisms involves upregulation of the neuroprotective protein, the presynaptic glutamate transporter 1 (GLT1), which removes glutamate from the synaptic cleft, thereby reducing its concentration in the synaptic cleft and thus decreasing the neurotoxic effects of glutamate [2]. It is well-established that excessive activation of the glutamatergic pathway plays an important role in the pathophysiology of MS; therefore, by enhancing the GLT1 expression, beta-lactam antibiotics might offer some degree of neuroprotection. Experiments in rodents and in human T-cells, both in vivo and in vitro, demonstrated that beta-lactam antibiotics modulate T-cell behavior, alter the T-cell gene expression and either upregulate or downregulate the pro-inflammatory T-cell phenotype via covalent binding to albumin [3].

Ceftriaxone (CEF), a beta-lactam drug, has been extensively studied as a neuroprotective agent against several glutamate-associated neurologic diseases due to its large volume of distribution [4]. In animal models of Parkinson’s and Alzheimer’s disease, CEF-treated animals showed improvement in memory impairment, downregulation of the tau protein, restoration of cognitive function, and neuronal density [5][6][7]. In a murine myelin–oligodendrocyte glycoprotein-induced (MOG) EAE model, CEF reduces the disease severity by causing a reduction in T-cell activation via the modulation of cellular antigen-presentation and impairment of antigen-specific T-cell migration into the CNS [7][8]. Despite these observations, CEF has not been tested in pwMS yet.

PCN, another beta-lactam antibiotic, has been studied in two trials in pwMS. A large UK-based case–control study reported that PCN administration of more than 2 weeks within 3 years prior to the appearance of the first symptom of MS was associated with a 50% reduced risk of developing MS [9]. On the contrary, a Danish case–control study of 3259 pwMS reported a higher risk of MS across a wide range of different antibiotics, including PCN, even after a short 7-day treatment course [10].

Interestingly, a more recent case–control study demonstrated allergy as a protective factor for MS. Patients with respiratory tract allergies were more likely to use antibiotics, including PCN, and those with other non-respiratory tract allergies also had a high likelihood of PCN use. Although no direct link was confirmed between PCN use and the risk of MS, PCN may mediate the relationship between allergies and MS. These results suggest that antibiotic use might not be a suitable indicator of bacterial infection to investigate the cause of MS [11]. These results were confirmed by a recent study that demonstrated no relationship between antibiotic exposure and the risk of MS [12].

# 2. Tetracyclines

## 2.1. Minocycline (Minocin, Dynacin, Ximino, and Solodyn)

Minocycline is a second-generation tetracycline that can cross the blood–brain barrier at clinically effective levels due to its lipophilic nature. Research on minocycline spans several decades, making it the most extensively studied antimicrobial. Minocycline showed a neuro-protective effect in several neurodegenerative diseases, such as Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, stroke, and MS [13][14][15]. The signaling mechanisms by which minocycline acts as a neuroprotectant in neurodegenerative diseases are complex, multiple, and mainly involve an anti-inflammatory, antiapoptotic, and anti-oxidative effect [16][17][18][19][20][21].

While the exact mechanisms of demyelination and axonal loss are not yet fully known, the literature supports that apoptosis in CNS neurons plays an important role in the pathogenesis of MS [22]. Minocycline is known to upregulate the expression of B-cell lymphoma 2, an anti-apoptotic protein, downregulate the expression of apoptosis-inducing factor, a pro-apoptotic protein, and stabilize the mitochondrial membrane to prevent the release of mitochondrial products from leaking out, thus preventing apoptosis [23][24].

The anti-inflammatory role of minocycline in MS is brought by the inhibition of T-cell migration and T-cell release of matrix metalloproteinases (MMPs) that damage the blood–brain barrier [25][26][27]. In a study where levels of different neurotrophic factors produced by peripheral blood mononuclear cells were measured, the levels of nerve growth factor (NGF) were significantly higher in patients who have fully recovered after an MS relapse [28]. Other observations showed that levels of brain-derived neurotrophic factor (BDNF) are lower in pwMS compared to healthy controls and increased post-relapse during the recovery period [29]. Using the EAE mouse model and performing experiments both in vivo and in vitro, Chen and colleagues demonstrated the upregulation of NGF and BDNF by minocycline [30].

These effects of minocycline have been evaluated across a wide range of animal models [31][32][33][34][35]. In the MOG-immunized rats, treatment with minocycline was found to reduce the T cell infiltration into the CNS and block the MMP-2 expression. Furthermore, minocycline-treated rats showed a delayed onset of the disease along with a dramatic reduction in disease activity and severity [31].

Minocycline administration in the EAE mouse model, after the disease onset, reduces mean and cumulative EAE scores by reducing T-cell infiltration, both CD4 and CD8, into the spinal cord without changing the cytokine profile [32]. Furthermore, it inhibited MMP activity, decreased MMP-9 production, and reduced the transmigration of T cells across a fibronectin matrix barrier [33]. In addition, minocycline was found to be efficacious in both mild and severe EAE in mice.

Early MS-related memory impairment is attributed to dental gyrus injury secondary to microglial activation [34]. In a mouse model of EAE, minocycline prevented dentate gyrus injury by inhibiting microglial activation [35]. In the same model, suboptimal doses of minocycline and hydroxychloroquine individually delayed the onset of clinical signs. Impressively, their combination markedly attenuated EAE severity until treatment was stopped. These results were expanded in the Biozzi ABH mice, a model of progressive MS, where the combination of minocycline and hydroxychloroquine successfully altered the chronic phase, which could potentially be beneficial and applicable in the progressive phases of MS in humans [36]. In a spectrometry-based proteomics analysis of an animal model of EAE, half of the minocycline-treated EAE animals exhibited no neurological symptoms on day 14, while the other half had neurological symptoms at the same time point [37]. In the rat model of MOGA-induced EAE, minocycline delayed disease onset, improved electrophysiological conduction of the optic system, rescued retinal ganglion cells (RGC), activated the anti-apoptotic pathways, decreased intraretinal glutamate levels, and decreased the severity of optic neuritis. Interestingly, after surgical transection of the optic nerve, minocycline increased RGC survival [38].

## 2.2. Doxycycline (Vibramycin D and Periostat)

Doxycycline is a tetracycline antibiotic that is lipid-soluble, so it highly penetrates the blood–brain barrier and acts directly on the CNS. It is rapidly and completely absorbed and is a strong MMP inhibitor [39]. While it has not been tested in animal models, it was evaluated in two clinical trials as an add-on therapy to interferon beta-1a. The first open-label trial of 7-month duration evaluated the efficacy and safety of a combination therapy consisting of intramuscular interferon βeta-1a and oral doxycycline in 15 RRMS participants with breakthrough disease. PwMS during the treatment period showed a reduction in the gadolinium-enhancing lesions, disability, and relapse ratio compared with the pre-treatment period. The combination was safe and well tolerated [40]. On the other hand, in a 6-month double-blinded trial of 60 pwMS, including both RRMS and active secondary progressive MS, no favorable MRI outcomes were observed when doxycycline was used as an add-on therapy to either subcutaneous or IM interferon β-1a. However, this combination was found effective in reducing relapses and improving EDSS scores [41].

Interestingly, in an experimental model of peripheral autoimmune neuritis, doxycycline effectively reduced peripheral inflammation to improve the outcome of this demyelinating disease of the peripheral nervous system,

suggesting that doxycycline may be considered a potential pharmacological agent for the management of neuropathies [42].

## 3. Rapamycin (Sirolimus)

Rapamycin, a macrolide antibiotic produced by Streptomyces hygroscopus, is an immunosuppressant and anti-cytoproliferative drug mainly used to prevent allograft rejection in organ transplantations [43]. The therapeutic effect of the drug in the CNS is due to its ability to cross the blood–brain barrier and exerts an anti-inflammatory effect [44]. In addition to its immunosuppressive effect, it inhibits apoptosis and activates intracellular autophagy via inhibition of the assembly of the subunits of the mammalian target of rapamycin (mTOR) [45][46][47]. The first step in the inhibitory molecular pathway includes inhibition of the mTOR kinase, which regulates the cell cycle; thus, rapamycin halts the antigen-mediated B- and T-cell proliferation [46][47][48]. In the relapsing- remitting EAE mouse model, administration of rapamycin at the disease peak or at the end of the first clinical attack has been shown to ameliorate the clinical course along with reduced demyelination and axonal loss via selective suppression of effector T cell function and expansion of T regulatory cells [48]. Similarly, in the chronic EAE mouse model, administration of the rapamycin to the already ill animals at the peak of their disease (therapeutic approach) ameliorated both clinical and histological manifestations of the disease, while early administration before the appearance of the symptoms (prophylactic approach) prevented the development of EAE. Moreover, it reduces the hyperalgesia by increasing the pain threshold, so it might have an additional benefit in the management of painful dysesthesias in pwMS [49]. Furthermore, in the same animal model, rapamycin was tested as an add-on treatment and was found to increase the immunomodulatory properties of the bone marrow-derived mesenchymal stem cells [50]. Another interesting phenomenon observed in the EAE animal model was the recovery of the gut microbiota to an almost normal level when the mice were treated with rapamycin in combination with MCC950. This observation suggests another mechanism of action for rapamycin, potentially beneficial in preventing and treating MS [51].

In pwMS, rapamycin was tested only in one pilot study, while its synthetic derivative, temsirolimus, was tested in a multicenter trial. In a single institution, single-arm study, 8 RRMS patients received a daily dose of rapamycin 2 mg over the 6-month study duration. After obtaining MRI scans and disability outcomes, participants showed a significant reduction in mean plaque area size and maximum area volume, while the minimum area volume decreased but not significantly. Half of the participants showed a non-significant decrease in the EDSS post-treatment, while for the other half, the EDSS remained unchanged. Moreover, genes encoding the T-regulatory cells were upregulated [52]. Temsirolimus, a synthetic derivative of rapamycin that is better absorbed than rapamycin, was tested in phase II, placebo-controlled study in 296 patients with RRMS and active secondary progressive MS. Participants were randomized to three different doses, and the highest dose of 8 mg daily showed a significant decrease of 51% in the number of relapses compared with the placebo group and a significant reduction in gadolinium-enhancing lesions. Despite the above results, a phase III study was not pursued due to the critical risk-to-benefit ratio, given the toxicity observed in the treatment group, consisting mainly of drug-induced hyperlipidemia, stomatitis, menstrual dysfunction, and rash [53][54].

## 4. Antivirals

Several viruses have been implicated in the pathogenesis and progression of MS, including EBV, cytomegalovirus (CMV), and varicella zoster virus (VZV) [55][56]. Among them, EBV, a herpesvirus that infects more than 90% of the global population, has been extensively studied over the last four decades, and its presence as a potential causative factor for the development of MS has been recently demonstrated [57][58]. The role of EBV in the early pathogenesis of MS is supported by several prospective studies demonstrating that elevated titers of EBV antibodies and EBV infection significantly increase the risk of developing MS later in life. Moreover, pwMS have greater than 99% EBV seropositivity as compared to non-MS patients, while the risk of MS is very low in patients who are EBV- seronegative [59][60][61][62][63]. The mechanism of EBV infection leading to MS onset is still debated. One possible mechanism is that EBV infects B-cells and causes these cells to grow, produce CNS antigen-specific antibodies, and enter the CNS to activate the T cells [64]. These T cells may also be activated by the small heat shock protein alpha B-crystallin induced by EBV in B cells and then recognize the alpha B-crystallin in glial cells in MS lesions [65]. Another possible mechanism is molecular mimicry: the cross-reaction of EBV antibodies with CNS-specific antigens, such as the myelin basic protein, to trigger the onset of the disease [66].

Lanz and colleagues reported that a monoclonal antibody produced from a B-cell clone derived from the CSF of an MS patient cross-reacted with the EBV nuclear antigen (EBNA-1) and GlialCAM, a protein found in CNS astrocytes and oligodendrocytes [67]. Further, these antibodies were also found in 20% of pwMS. Despite these findings, the main question is whether these antibodies are merely biomarkers or if they truly have a role in the pathogenesis of MS.

The B-cell depleting DMT mostly indiscriminately depletes or inhibits all B-cells; since these B cells would also include a subset of EBV-infected cells, these drugs may help lower the EBV load and hence the potential for EBV-induced immunopathological response in MS. For example, ocrelizumab treatment has been shown to reduce the EBV-specific immune response, indicating that the benefit of this drug in MS may be achieved via the removal of EBV antigenic stimulus [68].

Currently, no antiviral drugs are FDA-approved to treat EBV infection in vivo. There is anecdotal evidence that some antiretroviral agents that are also potent inhibitors of EBV lytic infection may suppress MS activity [69]. Interestingly, a comparative cohort study using administrative health databases with a cohort of 21,207 HIV-positive patients and 5,298,496 controls demonstrated a lower risk of developing MS in HIV-positive people compared with the control group, either due to the HIV-induced immunosuppression or to the antiretroviral medications use [70]. These findings led to the conduction of a phase 2a clinical trial investigating the effect of raltegravir (400 mg BID), an HIV integrase inhibitor, on 20 pwMS. The study duration was 6 months and consisted of a 3-month baseline phase followed by a 3-month treatment with raltegravir 400 mg twice a day. The primary outcome was the development of gadolinium-enhancing lesions in the treatment phase compared with the baseline phase, and secondary outcomes included quality of life, EBV shedding, EBV antigens, as well as immunological and inflammatory markers. After completion of the study, no change in the primary or secondary outcomes was reported [71]. Currently, an ongoing study investigates the effect of tenofovir alafenamide, another antiretroviral

agent, FDA-approved for chronic hepatitis B virus infection. The study explores its effect in combination with ocrelizumab or rituximab on MS-related fatigue, serum neurofilament light chains, disability, annualized relapse rate, EBV viral load, and titers (NCT04880577).

Several anti-herpetic drugs that inhibit EBV replication in vitro have been evaluated in EBV-related diseases. These antiviral drugs, including acyclovir, valacyclovir, and ganciclovir, inhibit EBV replication, but they lack efficacy over viral latent infection, which is the main factor in the MS pathogenesis. The investigators demonstrated that ganciclovir inhibits the proliferation of microglia and attenuates neuroinflammation. When given before the onset of the disease, it prevented the infiltration of T lymphocytes into the CNS and drastically reduced disease incidence and severity [72]. In pwMS, there are only a few published randomized and placebo-controlled clinical trials of anti-herpetic treatment with non-significant results. Most of these trials were conducted more than two decades years ago. One randomized, double-blinded, placebo-controlled trial investigated the role of acyclovir (800 mg TID) in 60 patients with RRMS [73]. Although acyclovir-treated patients showed 34% fewer exacerbations compared with placebo during the study duration, this difference has not reached statistical significance. However, in subsequent subgroup data analysis where patients were grouped according to exacerbation frequencies, acyclovir-treated patients had a reduced mean annualized relapse rate of 0.44, while the corresponding placebo group showed an increased annual exacerbation rate of 0.37 during the study ($p = 0.024$).

Two clinical trials investigated valacyclovir in pwMS. In a phase 2, placebo-controlled, double-blind, randomized study, Bech and colleagues investigated the effect of valacyclovir on the MRI-evident lesions in 70 RRMS with two or more relapses within 2 years before enrollment. Although valacyclovir treatment did not reduce the formation of new lesions, it had a significant effect on a subgroup of patients with high levels of disease activity. Valacyclovir-treated patients with more than one active lesion at baseline had 2.0 lesions per scan compared with 6.5 lesions per scan in the placebo group ($p = 0.025$), and the proportion of scans with no activity was 28% in the valacyclovir and 5% in the placebo group ($p < 0.001$). This study has mainly focused on MRI metrics and has not investigated any clinical outcome [74]. The second study was a double-blind, placebo-controlled trial of a 2-year duration. Fifty-eight pwMS were randomized to receive valacyclovir (3000 mg daily) or placebo for the study duration. The primary outcome was disease progression, and secondary outcomes included time to first attack, time to withdrawal, and attack rate. No significance was achieved, but some positive trends were noted in both primary and secondary outcomes. Similarly, no significant effect of the drug was demonstrated on MRI measures. Its safety profile was excellent, and no discontinuations were observed due to drug toxicity [75]. Furthermore, an ongoing study of 36-week duration, designed for pwMS on natalizumab, investigates the effect of famciclovir as an add-on therapy on EBV shedding in saliva, EBV serological markers, and EBV viral replication in blood (NCT05283551).

## 5. Hydroxychloroquine (HCQ)

In preclinical trials, this antimalarial drug was tested in the EAE animal model and was shown to reduce microglial activation and attenuate the severity of the disease [76]. Using in vitro cell culture assays, the mouse lysolecithin spinal cord model, and a combination of HCQ and indapamide, Brown et al. reported an attenuation in axonal injury, decrease in lipid peroxidation, and inhibition of microglia activity, therefore mitigating the substrates of

progression in MS [77]. In the mouse model of acute and chronic EAE, a combination of suboptimal doses of HCQ and minocycline suppressed clinical manifestations of EAE until treatment was stopped [36].

## 6. Other Antimicrobial Agents

Chlamydia pneumoniae is known to be present in the CSF in a subset of pwMS and has been hypothesized to even serve as a contributing factor in the disease pathogenesis [78]. Macrolides are antibiotics commonly used in the management of chlamydial infections. Despite their anti-inflammatory effects, animal studies are scarce with discouraging results. In the EAE mouse model, clarithromycin and azithromycin similarly inhibited nitric oxide (NO) production, NO synthase mRNA, and protein expression in vivo and in vitro and subsequently aggravated EAE [79].

In pwMS, the effect of azithromycin as a combination therapy with rifampin was tested in a single double-blinded, placebo-controlled trial of RRMS patients who showed Chlamydia pneumonia gene in their CSF with at least one gadolinium-enhancing brain lesion on baseline MRI scan. Participants were randomized to azithromycin and rifampin group or placebo. The primary outcome measure, the number of enhancing lesions, was not significantly affected by the combination therapy. However, in a post hoc analysis, three of four participants who received antibiotics showed a decrease in the volume of enhancing lesions. Moreover, the antibiotic group had significantly less parenchymal volume fraction loss compared to the placebo group [80].

In another study, 28 pwMS were recruited and were treated with three cycles of oral clarithromycin, 6 weeks each. Following the conclusion of the study, no significant difference was found in the EDSS and relapse rate between the treatment group and placebo [81].

The most recent development in the continuously evolving landscape of antimicrobials and MS is a placebo-controlled, currently enrolling trial exploring the effect of vancomycin on the gut microbiota composition, peripheral immune function, and MRI metrics (NCT05539729). The results of this trial will be available in 2025 and may improve our understanding of the interplay between antibiotics and MS.

Overall, several antimicrobials have been tested in animal models, resulting in an improved understanding of their complex mechanism of action. Clinical trials for some of the antimicrobials that have been completed showed excellent safety profile and good tolerability, while other clinical trials are still ongoing.

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