

Epigenetic Drugs for Multiple Sclerosis

Jacob Peedicayil*

Department of Pharmacology and Clinical Pharmacology, Christian Medical College, Vellore, India

Abstract: There is increasing evidence that abnormalities in epigenetic mechanisms of gene expression contribute to the development of multiple sclerosis (MS). Advances in epigenetics have given rise to a new class of drugs, epigenetic drugs. Although many classes of epigenetic drugs are being investigated, at present most attention is being paid to two classes of epigenetic drugs: drugs that inhibit DNA methyltransferase (DNMTi) and drugs that inhibit histone deacetylase (HDACi). This paper discusses the potential use of epigenetic drugs in the treatment of MS, focusing on DNMTi and HDACi. Preclinical drug trials of DNMTi and HDACi for the treatment of MS are showing promising results. Epigenetic drugs could improve the clinical management of patients with MS.



Keywords: DNA methyltransferase inhibitor, epigenetic, histone deacetylase inhibitor, multiple sclerosis.

INTRODUCTION

Multiple sclerosis (MS) is a chronic disorder of the central nervous system (CNS) involving demyelination, gliosis (scarring), and neuronal loss [1]. Over two and a half million people throughout the world are afflicted with the disease which makes it the most common reason for permanent neurological disability in young adults [1,2]. MS is about three times more prevalent in women than in men with recent evidence showing that the incidence of MS is on the rise [3]. The age of onset is usually between 20 to 40 years, with the average age of onset a few years earlier in women as compared to men [1, 2]. The prevalence of MS demonstrates a geographical variation with a higher prevalence in temperate regions. Increased prevalence also correlates with a higher socio-economic status [4]. Caucasians are more likely to develop MS than Africans or Asians, even when living in a similar environment [1].

The precise cause of MS remains unclear [5]. The most commonly accepted hypothesis for the pathogenesis of MS is that it begins as an inflammatory autoimmune disorder involving autoreactive lymphocytes (predominantly T-cells) to myelin basic protein and myelin oligodendrocyte glycoprotein [6]. MS is also thought to be dominated by microglial activation and chronic neurodegeneration [7]. The role of genes in the development of MS is suggested by the fact that there is a 5% concordance rate among same-sex dizygotic twins and 20 to 30% concordance rate among monozygotic twins [8]. The major histocompatibility complex (MHC) on chromosome 6 is the most strong MS susceptibility region in the genome [8]. Whole genome association studies have now identified more than 50 potential MS susceptibility genes, of which the DRB1*15:01 locus has been found to be the most significant [1].

Environmental factors that have been shown to play a role in development of MS include vitamin D, sunlight (UV-B radiation) and the Epstein-Barr virus [8]. The distinguishing feature of MS are multiple focal areas of myelin loss within the CNS termed lesions or plaques [9]. These plaques are believed to arise due to a break in the integrity of the blood-brain barrier in an individual who is genetically predisposed [10]. Due to the break, "if lymphocytes programmed to recognize myelin antigens enter the CNS, they may cause several events leading to the formation of an acute inflammatory demyelinating lesion" [10]. "Such lesions usually develop in the CNS white matter where the main targets are the myelin sheath and the myelinating cell, the oligodendrocyte" [10]. "Lesions are seen in all parts of the CNS but mainly involve optic nerves, subpial spinal cord, brainstem, cerebellum, and juxtacortical and periventricular white matter regions" [11]. "Although it has been suggested that MS is a disease mainly involving CNS white matter, recent pathologic and imaging studies have confirmed that demyelinated lesions are also commonly found in the cortical gray matter of MS patients" [11-14]. "Histologically, many basic processes promote the formation of plaques: inflammation, myelin breakdown, astrogliosis, oligodendrocyte injury, neurodegeneration and axonal loss, and remyelination" [15].

The clinical presentation of MS is very variable. The common symptoms of the disease comprise sensory disturbances in the face or the limbs, visual loss due to optic neuritis, diplopia resulting from internuclear ophthalmoplegia, acute or subacute motor weakness, limb ataxia, gait and balance difficulties, vertigo, sexual and bladder dysfunction, generalized fatigue and pain [1, 7]. Less commonly, patients present with psychiatric symptoms [16, 17], early dementia, convulsions and specific cortical deficits (aphasia, apraxia, alexia or neglect). The clinical course of MS presents as remissions and relapses and can be grouped into four categories: relapsing/remitting MS, which is the most common; secondary progressive MS; primary progressive

*Address correspondence to this author at the Department of Pharmacology and Clinical Pharmacology, Christian Medical College, Vellore, India; Tel: 91-0416-2284237; Fax: 91-416-2284237; E-mail: jpeedi@cmcvellore.ac.in

MS, the second most common form; and progressive/relapsing MS [1].

MS is usually diagnosed clinically [1]. Magnetic resonance imaging (MRI) is the investigation of choice to corroborate the clinical diagnosis [18]. The main requirement for the diagnosis of MS is the demonstration of CNS lesion dissemination in time and space, based upon either clinical findings alone or a combination of clinical and MRI findings [19, 20]. Cerebrospinal fluid analysis and measurement of evoked potentials in afferent (visual, auditory, and somatosensory) or efferent (motor) CNS pathways are useful additional procedures for confirming the diagnosis of MS [1].

CURRENT PHARMACOTHERAPY OF MULTIPLE SCLEROSIS

An important aspect of the pharmacotherapy of MS is the treatment of the acute episode which could be the first attack or an acute exacerbation of the chronic disease. Such episodes are usually treated with glucocorticoids. Three to seven day courses of methylprednisolone administered intravenously (0.5 to 1 gram daily), with or without a short prednisolone taper, are usually used [21, 22], although adrenocorticotropic hormone (ACTH) therapy has been found to be equally effective [23]. The second aspect of pharmacotherapy is the use of disease-modifying drugs that

reduce the activity of the underlying disease process in MS. Although these drugs do not cure the disease, they can potentially reduce disease activity and progression. They also shorten the duration of acute exacerbations, decrease their frequency, and provide symptomatic relief. Eight such drugs have been approved by the Food and Drug Administration (FDA) of USA for clinical use: four beta interferon drugs, glatiramer acetate, mitoxantrone, natalizumab and fingolimod. Even though it is known that these drugs act by exerting immunomodulatory effects, the exact mechanisms of action of these drugs in MS are still unclear [24, 25]. The earlier the patient is started on these drugs, the more effective they are in preventing relapses of disease [24]. However, they are also associated with major adverse drug reactions like alopecia, amenorrhoea, infections, malignancy, and cardiac toxicity. Other drugs which are not FDA-approved like azathioprine, methotrexate, cyclophosphamide, mycophenolate mofetil and cladribine are also commonly used by physicians off label. The third aspect of pharmacotherapy in patients with MS is symptomatic relief. Drugs for the management of pain, spasticity, spasms, depression, bladder and sexual dysfunction, and urinary tract infections are used. To take but one example, dalfampridine, which was only recently approved by the FDA, has been shown to improve walking speed in MS patients [24]. More details of the currently used drugs for the treatment of MS are given in Table 1.

Table 1. Current pharmacotherapy of multiple sclerosis.

Drug		Drug Class
Therapy for Acute Attacks		
Methylprednisolone		Glucocorticoid
ACTH		Anterior pituitary hormone
Disease-Modifying Drugs		
Interferon beta-1a (Avonex)		Cytokine
Interferon beta-1a (Pfizer)		Cytokine
Interferon beta-1b (Bayer)		Cytokine
Interferon beta-1b (Novartis)		Cytokine
Glatiramer acetate		Peptide
Mitoxantrone		Anthracenedione anticancer drug
Natalizumab		Monoclonal antibody
Fingolimod		Immunosuppressive drug
Symptomatic Therapy		
Analgesics, antispasmodics, antidepressants,		
Off-label Drugs		
Azathioprine		Purine analog
Methotrexate		Folic acid analog
Cyclophosphamide		Alkylating agent
Mycophenolatemofetil		Immunosuppressive drug
Cladribine		Purine analog

ROLE OF EPIGENETICS IN THE DEVELOPMENT OF MULTIPLE SCLEROSIS

Many lines of evidence suggest that abnormalities involving epigenetic mechanisms of gene expression contribute to the pathogenesis of MS: 1. MS is known to be a common, complex disease in which many genes and environmental factors are known to contribute to disease pathogenesis. In such diseases, it is thought that epigenetic mechanisms of gene expression are involved in disease pathogenesis [26, 27]. 2. In several common diseases, including MS, it has been difficult to find the underlying genes by genetic mapping studies, resulting in the term “missing heritability” of such diseases. The involvement of epigenetic mechanisms of gene expression in the pathogenesis of such diseases may be one explanation for the missing heritability [28, 29]. 3. The concordance rate for MS in identical twins, who are genetically identical, is only about 33%, suggesting that there are non-genetic factors in disease pathogenesis [1]. 4. Epigenetic mechanisms of gene expression are known to be involved in the physiological functions of T lymphocytes like the production of various cytokines [30]. 5. Epigenetic mechanisms are thought to be involved in parent-of-origin effects, a phenomenon in which the phenotypic effect of an allele depends on whether it is inherited from the mother or father [31]. Such effects have been demonstrated at the MHC in patients with MS [31]. 6. The clinical course of MS is very variable and characterized by remissions and exacerbations, suggesting the involvement of epigenetic mechanisms in its pathogenesis [32]. 7. Epigenetic mechanisms could help explain the difference in prevalence of MS between males and females [33]. 8. There is considerable evidence that epigenetic mechanisms are involved in promoting autoimmune diseases [34].

Several studies have been conducted on the involvement of epigenetic mechanisms in the pathogenesis of MS, and more are ongoing [35-38]. These studies have investigated changes in DNA methylation, histones, and microRNAs (miRNAs). Some studies have been conducted on brain cells (Table 2). Mastronardi *et al* [39] found that there was increased citrullination of histone H3 in normal appearing white matter of patients with MS in comparison with control subjects. Pedre *et al* [41] using chromatin immunoprecipitation, quantitative PCR, and immunohistochemistry, found a shift towards histone acetylation in the white matter of the frontal lobes of patients with chronic MS. Huynh and colleagues

[43] found that genes regulating oligodendrocyte survival like *BCL2L2* and *NDRG1* were hypermethylated and expressed at lower levels in MS-affected brains compared to controls, while genes related to proteolytic processing like *LGPN* and *CTSZ* were hypomethylated and expressed at higher levels. Tegla *et al* [44] investigated the expression of SIRT1, a member of the histone deacetylase (HDAC) class III family, and that of HDAC3 in PBMCs obtained from patients with relapsing-remitting MS. They found a significant decrease in the expression of SIRT1 but not that of HDAC3 in the PBMCs of the MS patients during relapses compared with controls and stable MS patients.

Studies have also been conducted focusing on epigenetic mechanisms in peripheral cells of MS patients. Cox *et al* [47] found using miRNA microarray analysis that the miRNAs miR-17 and miR-20a are under-expressed in the whole blood of patients with MS. This finding was confirmed by RT-PCR. The authors also demonstrated that these miRNAs modulate T cell activation genes in a knock-in and knock-down T cell model. Kumagai *et al* [49] found using bisulfite sequencing that over a third of subjects with MS had increased promoter methylation of the gene encoding the protein tyrosine phosphatase SHP-1, a negative regulator of pro-inflammatory signaling and autoimmune disease. More recently, Graves and co-workers [50] performed a genome-wide DNA methylation analysis of CD4+ T cells from 30 patients with relapse-remitting MS and 28 healthy controls using illumina 450K methylation arrays. They found evidence for association of DNA methylation at the HLA-DRB1 locus with risk for MS. Calabrese *et al* [51] studied the expression of enzymes involved in DNA methylation/demethylation in PBMCs from 40 subjects with MS and 40 matched healthy controls. The authors also performed DNA methylation analysis of specific promoters and analyses of global levels of 5-methylcytosine and 5-hydroxymethylcytosine (5hmC). They found that the expression of the DNA methylation-associated enzymes TET2 and DNMT1 was significantly reduced in PBMCs of MS subjects and that this was associated with aberrant methylation of the promoters of the genes encoding these enzymes. Moreover, 5hmC was reduced in PBMCs from MS subjects, probably as a result of reduced TET2 levels. More details on the findings of epigenetic studies in brain cells of MS patients are given in Table 2, and those from peripheral cells of MS patients are given in Table 3.

Table 2. Epigenetic studies in brain cells in multiple sclerosis.

Epigenetic Change	Tissue	Refs.
Increased citrullination of Histone H3	Normal appearing white matter	[39]
Up-regulation of miR-34a, miR-155, and miR-326	Astrocyte cultures	[40]
Enriched acetyl-histone H3	Frontal lobe white matter	[41]
Highly expressed miR-338, miR-155, and miR-491	Cerebral white matter	[42]
Genome-wide differences in DNA methylation	Pathology-free brain regions	[43]
Decreased expression of SIRT1	Active brain lesions	[44]

Abbreviations: miRNA = microRNA

TRIALS OF EPIGENETIC DRUGS FOR MULTIPLE SCLEROSIS

The considerable research going on in epigenetics has had an impact on pharmacology, leading to a new sub-speciality in pharmacology called pharmacoeugenetics [52]. The use of pharmacoeugenetics on a genome-wide basis is termed pharmacoeugenomics [52, 53]. A major aspect of pharmacoeugenetics and pharmacoeugenomics is epigenetic therapy, the use of drugs to correct epigenetic defects [54, 55]. Several categories of epigenetic drugs are being investigated. These include inhibitors of DNA methyltransferase (DNMTi), inhibitors of histone deacetylase (HDACi), histone acetyltransferase inhibitors (HATi), histone methyltransferase inhibitors (HMTi), and drugs targeting microRNAs (miRNAs). Among these different classes of epigenetic drugs, at present, most work is being done on DNMTi and HDACi. For identifying effective epigenetic drugs for MS, laboratory models of MS are required for assessing the potency and efficacy of these drugs. Laboratory models of MS have been available for over 75 years. These models have contributed to the development of the currently used drugs for MS. They comprise immune-mediated, toxic, viral and genetic models using mice and cell cultures [56]. Among the laboratory MS models, experimental autoimmune encephalomyelitis (EAE) in mice has been very widely used [56].

Table 4 summarizes the studies conducted to date involving trials of epigenetic drugs for the treatment of MS. As shown in Table 4, so far only preclinical drug trials have been conducted, and only a few have been conducted. Three studies have included the use of HDACi in preclinical trials of MS. Camelo *et al* [57] showed that the HDACi trichostatin A reduced spinal cord inflammation, demyelination, neuronal and axonal loss and ameliorated disability in the relapsing phase of EAE in mice. It also up-regulated antioxidant, anti-excitotoxicity and pro-neuronal growth and differentiation mRNAs [57]. It also inhibited caspase (cysteine-dependent aspartate directed proteases, a group of cysteine proteases which are essential in apoptosis, necrosis, and inflammation) stimulation and down-regulated gene targets of the pro-apoptotic E2F transcription factor pathway [57]. Kalinin *et al* [58] showed that “dimethyl fumarate (DMF) modified expression of HDACs in primary rat astrocyte culture. After 4 hours of incubation, levels of HDACs 1, 2, and 4 mRNAs were increased by DMF. After 24 hours the levels returned to or were below control levels”[58]. Astrocyte stimulation using pro-inflammatory cytokines significantly raised mRNA levels of HDAC after 24 hours. However, protein levels were not raised at that time. When cytokines were present, DMF reduced HDAC mRNAs, proteins, and activity. Proteomic analysis of DMF-treated astrocytes identified 8 proteins in which lysine acetylation was raised by DMF, including histones H2a.1 and H3.3. Ge *et al* [59]

Table 3. Epigenetic studies in peripheral cells in multiple sclerosis.

Epigenetic Change	Tissue	Refs.
Decreased expression of SIRT1	PBMCs	[44]
DNA methylation of MHC2TA shows no change	PBMCs	[45]
No change in DNA methylation	Lymphocytes	[46]
Under-expressed miR-17 and miR-20a	Whole blood	[47]
DNA methylation-dependent PAD2 up-regulation	PBMCs	[48]
Hypermethylation of promoter of SHP-1 gene	Leukocytes	[49]
Methylation differences at HLA-DRB1	CD4+ T cells	[50]
Down-regulation of TET2 and DNMT1 expression	PBMCs	[51]

DNMTi = DNA methyltransferase inhibitor; PBMCs = Peripheral blood mononuclear cells

Table 4. Trials of epigenetic drugs in multiple sclerosis.

Drug	Class	Model	Refs.
Trichostatin A	HDACi	Murine EAE	[57]
Dimethyl fumarate	HDACi	Astrocytes	[58]
Vorinostat	HDACi	Murine EAE	[59]
Decitabine	DNMTi	Cell culture	[60]
Decitabine	DNMTi	Murine EAE	[61]

DNMTi = DNA methyltransferase inhibitor; EAE = Experimental autoimmune encephalitis; HDACi = Histone deacetylase inhibitor

showed that the HDACi vorinostat reduced human CD14(+) monocyte-derived dendritic cell (DC) differentiation, maturation, endocytosis, and decreased DCs' stimulation of allogenic T cell proliferation *in vitro*. Furthermore, "it reduced DC-directed Th1-(Type1T helper) and Th17-polarizing cytokine production. Vorinostat also reduced Th1- and Th17-mediated EAE by minimizing CNS inflammation and demyelination. Th1 and Th17 cell functions were inhibited in EAE mice treated with vorinostat [59]. Vorinostat also decreased expression of co-stimulatory molecules of DC in mice" with EAE [59].

DNMTi have also been investigated in preclinical trials of epigenetic drugs in MS. Börner and co-workers [60] using quantitative RT-PCR showed that cannabinoid and opioid receptors are epigenetically regulated in SH SY5Y cells, which endogenously express μ opioid receptors and CB1, but not CB2, cannabinoid receptors. The authors showed that treatment of these cells with the DNMTi decitabine led to de novo induction of CB2, while mRNA levels of CB1 and μ opioid receptors were unaltered. In comparison, the treating of Jurkat lymphocytes, which endogenously express CB2, but not CB1 and μ opioid receptors, led to de novo induction of CB1 and μ opioid receptors, while mRNA levels of CB2 were not altered significantly [60]. These data are of interest because activation of cannabinoid and opioid neuronal pathways in MS is thought to be beneficial in the treatment of this disorder. More recently, Mangano *et al* [61] showed in two models of EAE using mice that decitabine significantly ameliorated the clinical and histological characteristics of EAE. These effects were found in prophylactic and therapeutic regimens of this drug. There also were raised transcript levels of anti-inflammatory cytokines and reduced mRNA expression of pro-inflammatory mediators [61]. In addition, treatment with decitabine increased the proportion of circulating regulatory T cells by inducing Foxp3 (a protein involved in immune responses and thought to function as a master regulator in the development and functioning of regulatory T cells) expression by demethylating a CpG island in the gene encoding Foxp3. The potential use of HDACi in the treatment of MS has been well reviewed in three articles [62-64].

IMPLICATIONS OF TRIALS OF EPIGENETIC DRUGS FOR MULTIPLE SCLEROSIS

Although not many preclinical trials of epigenetic drugs for treating MS have been conducted, as discussed above, the few that have been conducted have shown promising results. More preclinical trials of epigenetic drugs are warranted. The currently-available DNMTi [65] and HDACi [66] suffer from lack of specificity since they inhibit many isozymes instead of one or two isozymes. More isozyme-specific DNMTi and HDACi are needed. In addition to this, research on the role of epigenetic abnormalities in MS is presently in its early stages. More needs to be learnt about the exact abnormalities in epigenetic mechanisms of gene expression in MS, and how these abnormalities contribute to the pathogenesis of MS. Such knowledge would enable more targeted epigenetic therapy in the treatment of MS. In addition to DNMTi and HDACi, other classes of epigenetic drugs like HATi, and HMTi could in the future be

investigated in preclinical trials for the treatment of MS. As mentioned above, patients with MS sometimes have psychiatric symptoms and dementia. Epigenetic drugs are being evaluated for these conditions [67, 68]. Hence, in the future, epigenetic drugs could be of help in the management of psychiatric symptoms and dementia in MS patients.

CONCLUSIONS

MS is an important neurological disorder whose exact cause is still unclear despite decades of research. There is accumulating evidence that abnormalities of epigenetic mechanisms of gene expression could contribute to the pathogenesis of MS. There are a number of effective drugs currently available for the treatment of MS. Since abnormal epigenetic mechanisms may underlie MS, epigenetic drugs may also prove to be useful in the treatment of MS. Hence, epigenetic drugs could be a novel therapeutic option in MS treatment. However, much more needs to be learnt about the role of epigenetics in MS pathogenesis and more work is required in investigating the preclinical use of epigenetic drugs for treating MS before we can consider conducting clinical trials of these drugs for the treatment of MS.

CONFLICTS OF INTEREST

The author has no conflicts of interest. There were no funds used for this work.

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