Pharmacology Discovery Services

A Eurofins Discovery Partner Lab

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White Paper

EAE Models for Preclinical Studies to Better Evaluate Therapies for Multiple Sclerosis

Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease that primarily affects the central nervous system (CNS). The disease occurs when the immune system mistakenly attacks and damages the myelin sheath, a layer of lipid substance that surrounds and protects nerve fibers in the CNS[1]. Myelin acts as an insulator, facilitating the transmission of nerve impulses between the brain and the rest of the body. As a result of this immune response, the myelin sheath becomes damaged and forms scar tissue, or sclerosis, which disrupts the transmission of nerve impulses along the affected nerve fibers. This can lead to a range of neurological symptoms, including muscle weakness, lack of coordination, blurred vision, fatigue, and cognitive impairment[2].

With over 2.8 million people worldwide affected by MS, Pharmacology Discovery Services (PDS) offers efficacy testing for MS research that includes a deepening understanding of the disease and developing treatments.

MS is a complex disease that can progress in different ways, depending on the pattern of relapse and remission. Relapsing-remitting MS (RRMS) is the most common form, characterized by periods of symptom exacerbation followed by periods of recovery. Primary progressive MS (PPMS) is less common and typically progresses steadily over time without remissions. Secondary progressive MS (SPMS) follows an initial relapsing-remitting course but later transitions to a more progressive phase, with or without relapses[3].

Adults in their late twenties and early thirties are most prone to the disease, with an average global age of diagnosis at 32. Women are more likely to develop the disease than men, by a 2:1 ratio.

MS also appears to be more common in regions located at higher latitudes, such as northern Europe and Canada, suggesting a potential role for Vitamin D deficiency in disease development[4].

While the exact cause of MS is unknown, it is believed to be a combination of genetic and environmental factors that trigger an autoimmune response. Genetic factors appear to contribute to MS susceptibility, as the disease tends to cluster in families. Several genes, including the human leukocyte antigen (HLA) genes, have been identified as potentially involved in MS development, and variations in these genes have been associated with an increased risk of developing the disease[2]. However, genetic factors alone do not explain the development of MS, as the disease is not directly inherited in a simple pattern. Environmental factors also appear to play a crucial role in MS development. Factors such as geographic location[4], exposure to infectious agents[5] and toxins, including cigarette smoke and solvents, have been implicated as potential triggers of MS Lifestyle factors, such as diet and stress, may also influence MS development and progression[6]. A better understanding of the complex interplay of genetic and environmental factors that are involved in the development of MS may lead to improved prevention and treatment strategies for this challenging disease.

Currently, there is no cure for MS, but there are several disease-modifying therapies available that can help slow the progression of the disease and manage symptoms. Treatment options include corticosteroids to reduce inflammation during relapses, immunomodulatory drugs to modify the immune response, and symptom-specific medications to manage individual symptoms[7]. Ongoing research into the causes and treatments of MS offers hope for better outcomes and improved quality of life for those affected by this challenging disease.

Molecular Events in MS

At the molecular level, MS is characterized by inflammation, demyelination, and axonal damage in the CNS.

The inflammation in MS is characterized by the infiltration of the CNS parenchyma with immune cells, particularly T cells and B cells, which recognize self-antigens, including myelin antigens, as foreign. This leads to the activation of these immune cells, as well as parenchymal elements, including astrocytes and microglia, and the production of pro-inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), interferon (IFN)-γ, IL-23, and IL-17. These cytokines contribute to the activation and recruitment of more immune cells to the CNS and further exacerbate the inflammatory response[8].

The recruitment of immune cells to the CNS is also mediated by chemokines, such as CCL2, CXCL10, and CXCL12, which act as chemoattractants for immune cells. These chemokines are produced by immune cells, glial cells, and endothelial cells and are upregulated in MS lesions[9].

Demyelination in MS involves the breakdown of the myelin sheath that surrounds axons, leading to axonal damage and dysfunction. This process is mediated by the activation of immune cells, particularly T cells and macrophages, and the release of toxic molecules, such as nitric oxide and reactive oxygen species, which damage the myelin sheath and axons[8].

Axonal damage in MS can lead to neuronal degeneration and permanent neurological deficits. The loss of axons also leads to the activation of glial cells, which further contributes to the inflammatory response and can exacerbate disease progression. Astrocytes can release pro-inflammatory cytokines and chemokines, while microglia can phagocytose myelin debris and release pro-inflammatory cytokines and reactive oxygen species[8].

In Vivo Models of MS

There are several different animal models of MS that have been used in research, each with its strengths and limitations. These models include experimental autoimmune encephalomyelitis (EAE), Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD), toxin-induced demyelination, and genetically engineered mouse models[10].

TMEV-IDD is another animal model of MS that uses a viral infection to induce CNS inflammation and demyelination. The model involves the injection of Theiler's murine encephalomyelitis virus, which infects and damages oligodendrocytes, the cells that produce myelin in the CNS. The immune system then responds to the viral infection and attacks the infected oligodendrocytes, leading to inflammation and demyelination in the CNS[13].

Toxic models of MS involve the administration of chemicals or toxins that induce demyelination in the CNS. One such model is the cuprizone-induced demyelination model, which involves the administration of cuprizone, a copper chelator, to mice in their diet, resulting in cell death of oligodendrocytes and subsequent demyelination in the CNS, as well as activation of astrocytes and microglia[14]. Another toxic model of MS is induced by lysolecithin, an activator of phospholipase A2. Upon local injection of lysolecithin, demyelination at the focal area arises from the primary toxic effects of detergent on the myelin sheaths and not from the secondary effects on oligodendrocytes[15]. Toxic models of MS have provided valuable insights into the process of demyelination and remyelination processes in the disease and have been used to test the efficacy of potential remyelinating agents. However, these models have limitations, including a lack of immune system involvement, which is a key feature of MS pathogenesis.

Genetically engineered mouse models involve the manipulation of specific genes involved in MS development and progression. One common transgenic mouse model of MS involves the manipulation of the myelin oligodendrocyte glycoprotein (MOG) gene. Mice that express MOG develop an autoimmune response against MOG and exhibit inflammation and demyelination in the CNS[16]. Transgenic mouse models can provide insights into the role of individual genes in the disease process and can be used to test the efficacy of new therapies targeted at specific pathways.

Experimental autoimmune encephalomyelitis (EAE) is the most widely used animal model of MS. It involves the injection of myelin antigens along with adjuvants to induce an autoimmune response in the animal, leading to inflammation and demyelination in the CNS[11]. The model has provided valuable insights into the pathogenesis of MS and has been used to test the efficacy of potential therapies that have been approved by the FDA, such as natalizumab, fingolimod, glatiramer acetate, alemtuzumab, and dimethyl fumarate[12].

Among these models, EAE is the most widely used due to several advantages:

1) EAE is a highly reliable and reproducible model that can be induced consistently in various animal species and strains, which makes it easier to study and compare results across different experiments.
2) It is relatively simple to induce and monitor, requiring the injection of myelin antigens along with adjuvants to induce an autoimmune response in the animal, leading to inflammation and demyelination in the CNS.
3) EAE can replicate some aspects of the human MS disease course, including the relapsing-remitting pattern or the progressive form of the disease, which allows researchers to study the underlying mechanisms and test the efficacy of new therapies.
4) Additionally, EAE models involve an autoimmune response, which makes it easier to study the immune system's role in MS development and progression.
5) EAE has also been shown to be a valuable predictor of the efficacy of therapies in human clinical trials, making it a valuable tool for drug development. While other animal models of MS have their own advantages, EAE's reliability, reproducibility, and ability to replicate some aspects of human MS have made it a popular choice for MS research[17].

Types of EAE Model

EAE can be achieved by two different procedures:

- A passive transfer, in which the myelin-specific T cells from a donor animal are transplanted into a recipient animal
- An active immunization, which could be induced by injecting myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG)

MBP-induced EAE models have been widely used in rodents to study the disease. However, the disease course in these models can be variable, and they may not replicate the clinical or histological features of human MS. PLP-induced EAE models, which have also been used in rodents, are characterized by a relapsing-remitting disease course that more closely resembles the clinical course of human RRMS than other EAE models. MOG-induced EAE models are characterized by a monophasic or chronic disease course that can mimic the clinical and pathological features of human PPMS[12,18].

The type of adjuvant used in EAE models can also affect the disease course and severity. For example, complete Freund's adjuvant (CFA) is a potent adjuvant that can induce severe EAE, while incomplete Freund's adjuvant (IFA) is a milder adjuvant that can induce a less severe disease course. Other adjuvants, such as pertussis toxin, can also be used to enhance disease severity and accelerate disease onset[19].

PDS offers efficacy testing using the MOG-induced EAE model, which has several applications in MS research, including studying the underlying mechanisms of the disease, identifying biomarkers, studying genetic and environmental factors, and developing and testing new therapies.

Procedure Summary

The animals, groups of 10 female 8- to 12-week-old C57BL/6 mice, will be immunized with MOG_{35-55}/CFA emulsion subcutaneously (SC) at two sites per animal (0.1 mL/site) on Day 0, as well as pertussis toxin (PTX, 0.1 mL) intraperitoneally (IP) on Day 0 and Day 1.

Following immunization, animals will develop a clinical disease that mimics many aspects of MS, including inflammation, demyelination, and axonal damage. The clinical signs of EAE typically include limb paralysis, ataxia, and loss of body weight. Disease severity is assessed using a standardized scoring system, such as the 0-5 scale developed by the National Multiple Sclerosis Society[20].

For efficacy testing, the vehicle, the test articles, and the positive control, fingolimod at 3 mg/kg, will each be administered orally once daily starting on Day 0 for a total of 21 days. Body weight and clinical score will be recorded daily during the study period (Figure 1). On Day 29, animals will be sacrificed after disease scoring and body weight measurement, and the blood sample will be collected for hematology analysis. Immediately after the blood collection, the brain and spinal cord samples will also be harvested for histopathological evaluation. Other measurements that can also be requested include behavioral testing, flow cytometric analyses of immune cells in the spleen, lymph nodes, or CNS tissues, and immunohistochemistry (IHC) etc.

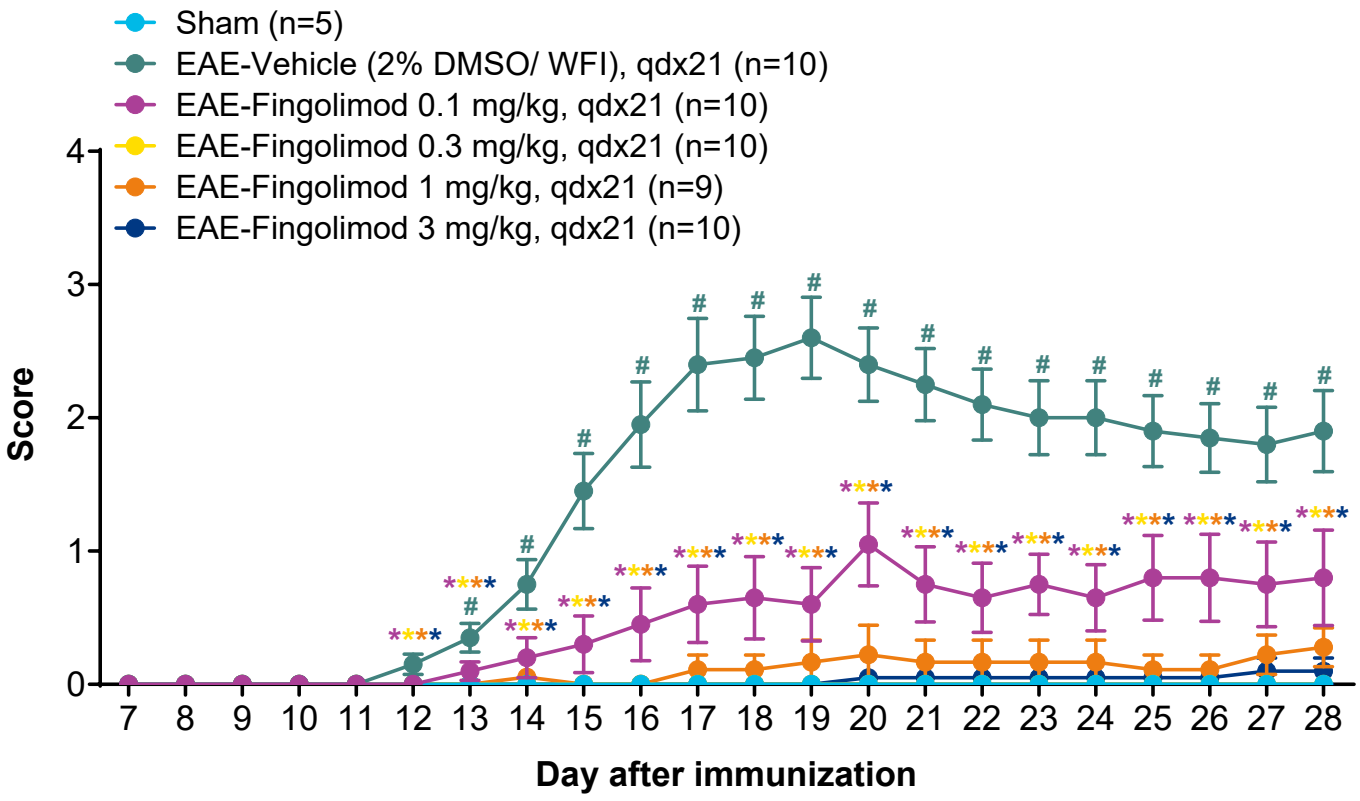

Figure 1. MOG-indued EAE model. In the EAE-vehicle control group, the disease onsets at around Day 12, and peaks at about Day 19. The repeated administrations of fingolimod dose-dependently and significantly relieves the disease progression.

Summary

Multiple Sclerosis (MS) is an autoimmune disease that affects the central nervous system, caused by the immune system attacking the myelin sheath surrounding nerve fibers, resulting in neurological symptoms such as muscle weakness, blurred vision, and cognitive impairment. MS has different progression patterns, with relapsing-remitting MS (RRMS) being the most common form. While the exact cause of MS is unknown, genetic and environmental factors are believed to trigger an autoimmune response. Treatment options include corticosteroids, immunomodulatory drugs, and symptom-specific medications. In animal models of MS, EAE is the most widely used, replicating some aspects of the human disease course, allowing researchers to study the underlying mechanisms of relapses and remissions, and test the efficacy of new therapies. Among EAE models, MOG-induced EAE has several applications in MS research, including studying the underlying mechanisms of the disease, developing, and testing new therapies, studying genetic and environmental factors, and identifying biomarkers. Ongoing research into the causes and treatments of MS offers hope for better outcomes and improved quality of life for those affected by this challenging disease.

References

1. Compston A, Coles A. Multiple sclerosis. *Lancet*. 2002 Apr 6;359(9313):1221-31. http://doi.org/10.1016/S0140-6736(02)08220-X. PMID: 11955556

2. Compston A, Coles A. Multiple sclerosis. *Lancet*. 2008 Oct 25;372(9648):1502-17. http://doi.org/10.1016/S0140-6736(08)61620-7. PMID: 18970977

3. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology*. 1996 Apr;46(4):907-11. http://doi.org/10.1212/wnl.46.4.907. PMID: 8780061

4. The Multiple Sclerosis Internationl Federation. Atlas of MS. 3rd ed, September, 2020.

5. Soldan SS, Lieberman PM. Epstein-Barr virus and multiple sclerosis. *Nat Rev Microbiol*. 2023 Jan;21(1):51-64. http://doi.org/10.1038/s41579-022-00770-5. PMID: 35931816

6. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. *Ann Neurol* . 2007 Jun;61(6):504-13. http://doi.org/10.1002/ana.21141. PMID: 17492755

7. McGinley MP, Goldschmidt CH, Rae-Grant AD. Diagnosis and Treatment of Multiple Sclerosis: A Review. *JAMA*. 2021 Feb 23;325(8):765-779. http://doi.org/10.1001/jama.2020.26858. PMID: 33620411

8. Frohman EM, Racke MK, Raine CS. Multiple sclerosis-the plaque and its pathogenesis. *N Engl J Med*. 2006 Mar 2;354(9):942-55. http://doi.org/10.1056/NEJMra052130. PMID: 16510748

9. Zhang GX, Baker CM, Kolson DL, Rostami AM. Chemokines and chemokine receptors in the pathogenesis of multiple sclerosis. *Mult Scler*. 2000 Feb;6(1):3-13. http://doi.org/10.1177/135245850000600103. PMID: 10694839

10. Procaccini C, De Rosa V, Pucino V, Formisano L, Matarese G. Animal models of Multiple Sclerosis. *Eur J Pharmacol*. 2015 Jul 15;759:182-91. http://doi.org/10.1016/j.ejphar.2015.03.042. PMID: 25823807

11. B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *Br J Pharmacol*. 2011 Oct;164(4):1079-106. http://doi.org/10.1111/j.1476-5381.2011.01302.x. PMID: 21371012

12. Robinson AP, Harp CT, Noronha A, Miller SD. The experimental autoimmune encephalomyelitis (EAE) model of MS: utility for understanding disease pathophysiology and treatment. *Handb Clin Neurol*. 2014;122:173-89. http://doi.org/10.1016/B978-0-444-52001-2.00008-X. PMID: 24507518

13. Tsunoda I, Fujinami RS. Neuropathogenesis of Theiler's murine encephalomyelitis virus infection, an animal model for multiple sclerosis. *J Neuroimmune Pharmacol*. 2010 Sep;5(3):355-69. http://doi.org/10.1007/s11481-009-9179-x. PMID: 19894121

14. Matsushima GK, Morell P. The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. *Brain Pathol*. 2001 Jan;11(1):107-16. http://doi.org/10.1111/j.1750-3639.2001.tb00385.x. PMID: 11145196

15. Jeffery ND, Blakemore WF. Remyelination of mouse spinal cord axons demyelinated by local injection of lysolecithin. *J Neurocytol*. 1995 Oct;24(10):775-81. http://doi.org/10.1007/BF01191213. PMID: 8586997

16. Anderson AC, Chandwaskar R, Lee DH, Sullivan JM, Solomon A, Rodriguez-Manzanet R, Greve B, Sobel RA, Kuchroo VK. A transgenic model of central nervous system autoimmunity mediated by CD4+ and CD8+ T and B cells. *J Immunol*. 2012 Mar 1;188(5):2084-92. http://doi.org/10.4049/jimmunol.1102186. PMID: 22279107

17. Ransohoff RM. Animal models of multiple sclerosis: the good, the bad and the bottom line. *Nat Neurosci*. 2012 Jul 26;15(8):1074-7. http://doi.org/10.1038/nn.3168. PMID: 22837037

18. Glatigny S, Bettelli E. Experimental Autoimmune Encephalomyelitis (EAE) as Animal Models of Multiple Sclerosis (MS). *Cold Spring Harb Perspect Med*. 2018 Nov 1;8(11):a028977. http://doi.org/10.1101/cshperspect.a028977. PMID: 29311122

19. Libbey JE, Fujinami RS. Experimental autoimmune encephalomyelitis as a testing paradigm for adjuvants and vaccines. *Vaccine*. 2011 Apr 12;29(17):3356-62. http://doi.org/10.1016/j.vaccine.2010.08.103. PMID: 20850537

20. Miller SD, Karpus WJ. Experimental autoimmune encephalomyelitis in the mouse. *Curr Protoc Immunol*. 2007 May; Chapter 15:15.1.1-15.1.18. http://doi.org/10.1002/0471142735.im1501s77. PMID: 18432984