

ICOPMAP SPECIAL EDITION

REVIEW

Neurotoxin-induced animal model of multiple sclerosis: Molecular mechanism focus

Yandi Permana¹ , Soni Siswanto² , Nur Hidayah Kaz Abdul Aziz³ , Bantari Wisynu Kusuma Wardhani^{1,4} 

¹ Faculty of Military Pharmacy, Republic of Indonesia Defense University, Bogor, West Java, Indonesia

² Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

³ School of Pharmaceutical Sciences, Universiti Sains Malaysia, Pinang, Malaysia

⁴ Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), Bogor, West Java, Indonesia

Keywords

Animal model
Multiple sclerosis
Myelin essential protein
Myelin oligodendrocyte glycoprotein

Correspondence

Bantari Wisynu Kusuma Wardhani
Faculty of Military Pharmacy
Republic of Indonesia Defense
University
Bogor
Indonesia
bantariwisynu@gmail.com

Abstract

Background: Neurotoxins can alter the central nervous system. They induce severe clinical symptoms such as encephalopathy, convulsions, muscle paralysis, and respiratory failure. Hence, the neurotoxin can mimic human central nervous system disorders such as Multiple Sclerosis (MS) to study pathophysiology and drugs of development. **Objective:** This mini-review compared the neurotoxins for mimicking MS in animal models. **Method:** This study was a narrative review using the scientific electronic databases Scopus, PubMed, and Google Scholar. All related articles by keywords animal models, multiple sclerosis, and neurotoxins were collected by YP and BW. All authors contributed to manuscript development. **Result:** Cuprizone, ethidium bromide, lysolecithin, Myelin Oligodendrocyte Glycoprotein (MOG), and Myelin Essential Protein (MBP) are neurotoxins for MS animal models. Cuprizone is particularly relevant for use in studies addressing toxic mechanisms of the demyelination process and studies of therapeutic interventions. Ethidium bromide causes spinal cord demyelination with distinctive features in the oligodendrocytes and astrocytes. However, it is a carcinogen agent. Lysolecithin is suitable for old animal models because the duration for remyelination is relatively longer than others. MOG and MBP are preferable to resemble MS pathophysiology in humans. **Conclusion:** MOG and MBP are appropriate for generating animal multiple sclerosis models for further in vivo experiments.

Introduction

The central nervous system (CNS) is essential to the body because it regulates all human activities, such as motor and sensory. The Central Nervous System comprises the human brain and spinal cord. The spinal cord is made up of 33 bones in the spine. The brain is housed in a particular bone called a cranium. It protects the brain from injury. A layer of meninges separates the cranium and brain. The cranium and brain prevent the brain from rubbing against the cranium (American Association of Neurological Surgeons, 2023). The brain has plasticity in its development. It proliferates during adolescence. The older the brain is, the more neurodegenerative it gets, but it can be overcome socially and at work (ET, 2007).

The brain processes the stimulus signals sent by the spinal cord. The spinal cord has two crucial parts, namely afferent and efferent. Afferent or sensory nerves carry signal transduction from the skin, joints, organs, and visceral nerves to the brain. Efferent or motor nerves carry signals from the brain to specific body parts (Ludwig & Das, 2023). The transmission of nerve signals involves electrical conductivity and chemical components. Electrical conductivity can occur because acetylcholine (a neurotransmitter) binds to its receptor (muscarinic). Activation of Na⁺/K⁺/ATPase channels results in a voltage difference, leading to signalling. (McLachlan, 2017).

Some toxins, further classified as neurotoxins, can alter CNS function. Neurotoxins are exogenic substances

that cause phenotypic lesions in dopaminergic neurons and kainite receptors. Endogenous substances are neurotoxins because they can induce apoptosis, necrosis, or necroptosis. In fact, exogenous is an imitation of endogenous, which has been used to study neurodevelopment and neurotrophin efficacy (Juan Segura-Aguilar, 2001). Phenotypic lesions and apoptosis result from neurotoxins interfering with neurotransmitter release, interfering with central dogma processes, initiating abnormal protein formation, and eliminating neurotrophic stimulation. The existence of tissue damage determines the functional repair of cells, the severity of the damage, and the presence of neuropathology in the CNS (poor prognosis) (Spencer & Lein, 2014).

Some neurotoxins are specific to their targets. 6-OHDA (6-hydroxydopamine) is more selective in dopaminergic and noradrenergic neurons because of its high affinity compared to other nerves. Neurotoxin binding to non-specific receptor subtypes does not affect binding to specific receptors. Neurotoxin binding reactions with non-specific receptors have little effect on the body's physiology. There are more non-specific than particular receptors, so a large amount of toxin is needed (Adams & Olivera, 1994). Each neurotoxin has a different latency time between exposure and effect. Latent time is associated with production and neurotoxic metabolic actions (Spencer & Lein, 2014). Some neurotoxins cause severe clinical symptoms such as encephalopathy, convulsions, muscle paralysis, and respiratory failure. This toxic effect is used for chemical weapons (Carota *et al.*, 2016). Despite the danger of its toxicity, this neurotoxin can be used to support drug development processes in preclinical trials. Neurotoxin mimics human central nervous system disorder as well as multiple sclerosis. However, animal models with neurotoxins cannot represent all elements of the disorder (Kostrzewa, 2009).

Multiple sclerosis (MS) is referred to as an autoimmune disease. Cytotoxic T cells that successfully infiltrate the blood-brain barrier bind to antigens presented by antibodies in the myelin sheath. As a result, the myelin sheath becomes permanently deformed (demyelinating). The impulse conduction process could be more optimal due to demyelination. The impacts are sensory disturbances, loss of control in striated muscles, loss of balance, and other disorders related to

the CNS. This defect is permanent because the cell cannot regenerate. Until now, the drug developed by researchers is not to treat multiple sclerosis. The therapy that has been growing so far is a treatment for sudden relapses (exacerbations). During this COVID-19 pandemic, multiple sclerosis is a significant disease to pay attention to. The SARS-CoV-2 virus triggers a cytokine storm, initiating autoimmunity. Autoimmunity increases the severity of myelin sheath deformation. Multiple sclerosis is becoming an increasingly strategic issue, considering that evolving therapies do not guarantee long-term effects.

Multiple sclerosis already has several FDA (Food and Drug Administration)-approved therapies. Treatment with beta interferon is the first therapy approved by the FDA (Selewski *et al.*, 2010). After that, glatiramer acetate was also approved as a treatment for multiple sclerosis. Intramuscular and subcutaneous administration routes carry out this treatment. Clinical trials have shown that administering beta interferon reduces the total exacerbation rate and the number of new lesions. However, the mechanism of action has not been identified, and nearly half of the patients experience depressive symptoms after using this drug (Goodkin, 1996). Glatiramer acetate can reduce T1-hypertensive and T2-hypertensive lesions. Glatiramer acetate reduced the level of disability as measured by the EDSS (Expanded Disability Status Scale) in 65.3% of the test group. The use of glatiramer acetate can form the presence of anti-glatiramer antibodies, which reduce the efficacy of glatiramer acetate. The mechanism of action of glatiramer acetate for multiple sclerosis is also not known (Tselis *et al.*, 2007). After conducting a research study, several new therapies for curing multiple sclerosis are dimethyl fumarate, teriflunomide, and fingolimod. The drug is used orally. These three oral drugs can reduce the percentage of defects and T2 lesions. Oral therapy agents have side effects that are more acceptable to patients than beta interferon and glatiramer acetate. However, this therapy does not yet have safety data for long-term use (Kretzschmar *et al.*, 2016). Therefore, therapy for multiple sclerosis still requires further research.

Furthermore, the National Health Service (NHS) of the United Kingdom (UK) has established a therapeutic option for multiple sclerosis. Table I presents this therapy based on the NHS.

Table 1: Multiple sclerosis based on the NHS

Multiple sclerosis phenotype	Therapy
Single clinical episodes without MRI (Magnetic Resonance Imaging) abnormalities allow the diagnosis of MS	No treatment (1)*
Single clinical episode with MRI abnormalities fulfilling the McDonald criteria for relapsing-remitting MS	No treatment (2)(3)* Interferon beta 1a or glatiramer acetate alemtuzumab or ocrelizumab
First-line therapy of relapsing-remitting multiple sclerosis (RRMS)	
RRMS: 2 significant relapses in the last two years	Interferon beta 1a and 1b Dimethyl fumarate (4)* Glatiramer acetate Teriflunomide Alemtuzumab or ocrelizumab (5)*
RRMS: 1 relapse in last 2 years AND radiological activity	Interferon beta 1a and glatiramer acetate (6)* Alemtuzumab or ocrelizumab (5)*
Rapidly evolving severe MS	Alemtuzumab or ocrelizumab (7)* Cladribine (7)* Natalizumab
Intolerance to first-line therapy	
RRMS: 2 significant relapses in the last 2 years	Interferon beta 1a and 1b (Extavia) Dimethyl fumarate Glatiramer acetate Teriflunomide Alemtuzumab or ocrelizumab
RRMS: 1 relapse in last 2 years AND radiological activity	Interferon beta 1a Glatiramer acetate Alemtuzumab or ocrelizumab
Rapidly evolving severe MS	Alemtuzumab or ocrelizumab Cladribine Natalizumab
Disease activity on second-line therapy	
Disease activity on second-line therapy	Alemtuzumab or ocrelizumab Cladribine Fingolimod
Rapidly evolving severe MS	Natalizumab Alemtuzumab or ocrelizumab Cladribine

*1. First-line trials have shown no long-term effect on the accumulation of disability.
 2. Interferon beta 1a and glatiramer acetate may be given to patients with relapse-remitting multiple sclerosis under the new diagnostic criteria.
 3. In conditions of poor prognosis, such as rapidly progressing permanent disability, alemtuzumab or ocrelizumab may be administered—further monitoring in the use of this therapy.
 4. Dimethyl fumarate is more effective than beta-interferon, glatiramer and teriflunomide.
 5. In RRMS, alemtuzumab has a higher risk, so it needs patient approval and intensive monitoring.
 6. Interon-beta and glatiramer acetate are allowed for patients with significant relapses and are closely monitored with MRI results.
 7. Alemtuzumab/ocrelizumab and cladribine are safer than natalizumab.

In the early inflammatory phase of multiple sclerosis, treatment based on disease modification has high efficacy and is optimal. The therapy used is Bruton tyrosine kinase. Tyrosine kinase is an essential therapy for progressive multiple sclerosis. Therapies targeting remyelination have failed to demonstrate conclusively a comprehensive therapeutic benefit. With myelin repair, the manifestation obtained is an improvement in clinical symptoms. Targeting chronic Epstein-Barr

virus and microbiome dysbiosis reflects an association of microbial risk factors for multiple sclerosis and therapeutic interventions.

The drug discovery in multiple sclerosis requires a representative animal model to assess its efficacy and toxicity. Three animal models are used for MS: autoimmune encephalomyelitis (EAE), murine Theiler's encephalomyelitis virus (TMEV), and neurotoxins. The neurotoxin model is less representative of MS than

TMEV and EAE. Toxins, such as lysolecithin and cuprizone, only induce the demyelination process, which is insufficient to be an animal model of multiple sclerosis. The pathophysiological mechanism has yet to be elucidated. Therefore, this review aims to discuss neurotoxins in animal models that are appropriate to represent various sclerosis diseases.

Methods

This study was a narrative review using the scientific electronic databases Scopus, PubMed, and Google Scholar. YP and BW collected all related articles by keyword animal models, multiple sclerosis, and neurotoxins. All authors contributed to manuscript development.

Results

Central nervous system

The mammalian brain is divided into the cerebral cortex, cerebellum, striatum, and diencephalon. *Loxodonta africana* (African elephant) is a mammal with an enormous brain weight. The brain's weight reaches 4000 grams. The number of constituent neuron cells ranges from 36 million to 257 billion. In most species of mammals, many neuron cells are deposited in the cerebellum with a percentage of 53-98%, while in RoB (rest of the brain), the number of neuron cells is small (Herculano-Houzel, 2012). The forebrain is the largest and has complex functions. The forebrain plays a significant role in regulating thinking and speaking activities. The forebrain is also responsible for perceptual processing, memory, speech, and thought. It is divided into two parts, namely the thalamus and the hypothalamus. It is divided into two parts, namely the thalamus and the hypothalamus.

The thalamus is between the telencephalon and the brainstem. The thalamus actively sends information to the cortex and receives it from the senses (Serlin et al., 2015). The third ventricle divides the thalamus into two parts (right and left). In addition, there is also a section of the medulla in the brain. The medulla comprises fibres and several nerve cells that help regulate heart rate reflexes, blood pressure, and relay stations. The medulla has many fibre tracts that convey information from nerve cells and the spinal cord. The cerebrum is divided into several sections: the frontal, occipital, temporal, and parietal.

The frontal lobe regulates motor functions, facilitates language abilities, and oversees various cognitive processes (Chayer & Freedman, 2001). The frontal lobe

regulates memory, mood, self-awareness, and personality. Writing activity and speaking skills are also regulated by the frontal lobes in Broca's area. The occipital lobe can interpret visual information located in the visual cortex. The temporal lobes can help humans understand language and writing (Lu et al., 2002). The parietal lobe translates auditory, visual, motor, sensory, and memory information. The cerebellum can maintain the precision and accuracy of motor activity by controlling the coordination of voluntary movements.

The brain size is 3500 cm³. It generates a power of 15 watts. The power provokes heat to drive the blood flow. The development of brain power requires increased nervous organisation, signalling processing, and thermodynamics simultaneously (Hofman, 2014). The brain begins to experience embryological development for three weeks. It was exposed to white proteins and phospholipids. These proteins and phospholipids form myelin. In addition, the brain also has grey parts because there is a collection of neurons and glial cells (Taylor et al., 2019).

Nerve grooves form when the neural plate is folded. Nerve pathways are responsible for the formation of CNS structures. Brain vesicles consist of two types: primary and secondary. The forebrain, midbrain, and hindbrain are the primary brain vesicles. The secondary brain is the result of differentiation from the primary brain. The hindbrain differentiates into the mesencephalon and myelencephalon, while the forebrain becomes the telencephalon and diencephalon (Thau et al., 2022).

The term vesicle is taken from the Greek word whose root is encephalon (en- = inside; cephalon = head). The first few syllables usually indicate the location of the developing nervous system, for example, the prosencephalon, mesencephalon, and rhombencephalon. Pros- means "in front of". Mes- means "in the middle". Rhomb- means a geometric figure with four equal sides. The telencephalon will develop into a cerebrum. The diencephalon will grow into the thalamus and hypothalamus. The embryo's diencephalon will undergo structural changes to become the retina. It is rare because the inner structures can differentiate into fully formed peripheral structures. The mesencephalon does not undergo differentiation. The stable midbrain does not experience the primary vesicle development stage. The part of the brain around the midbrain undergoes a stage of differentiation that divides the brain into the forebrain, midbrain, and hindbrain. The rhombencephalon differentiates into the metencephalon (brain stem) and myelencephalon (medulla oblongata). The cerebellum accounts for

about ten percent of the mass in the brain. The brainstem connects it to the rest of the brain (OpenStax College, n.d.). The brainstem and cerebellum are differentiated into the same vesicles. The processes collectively described are presented in Table II.

Table II: The development of secondary vesicles in different brain parts

Brain parts	Brain parts differentiation
Telencephalon	Cerebral cortex, basal ganglia (caudate, putamen, globus pallidus), basal forebrain nuclei, hippocampus
Diencephalon	Thalamus, hypothalamus, posterior pituitary, retina
Mesencephalon	Midbrain
Metencephalon	Pons, cerebellum
Myelencephalon	Medulla

Thermoregulation in the brain is carried out by forming micelles (lipids in water). The formation of micelles in the brain can cause the phenomenon of the second law of thermodynamics. The existence of the laws of thermodynamics makes the process of exchanging information happen. Brain cell membrane cholesterol can form a lipid framework for receptor-mediated cell-cell signalling (Torday & Miller, 2016). In all mammals, the growth of neuron cells in the brain is called neurogenesis. Adult neurogenesis in the mammalian brain occurs in the hippocampus and Subventricular Zone (SVZ). Neural stem cells (NSC) in the hippocampus develop into mature granule cells. In the subventricular zone, progenitor cells (cells that differentiate into specific cells) proliferate along the rostral migratory stream (RMS) toward the Olfactory Bulb (OB). These cells form olfactory interneurons. Granular cells are formed by expressing SRY-related HMG-box (Sox) and GFAP by NSC types 1 and 2. As a result, active neuroblasts proliferate to form immature neurons. Immature neurons continue to express Doublecortin (DCX) until they form granule cells. The formation of olfactory interneurons starts from type c cells in SVZ, which continue to increase and express *Ascl1* (achaete-scute homolog 1). The process proliferates progenitor cells toward the OB (Ming & Song, 2011). The existence of adult neurogenesis functions for memory formation. Almost every memory mammal is formed in the cerebellum (Shackelford, 2017).

The human/mammalian central nervous system comprises neuron cells that initiate the development of thought, language, and memory. Neurons, or nerve cells, are the most extended cells in the body and can transmit nerve impulses throughout the body (Gautam,

2017). Neurons are connected through synapses, which deliver neurotransmitters to the inter-synaptic gap. Neurons have three classifications based on their function: sensory neurons, relay neurons, and motor neurons (Olivia Guy-Evans, 2023). Neurons are considered mini calculators or computing devices because they add and subtract voltages. Neurons comprise three main structures: the dendrites, the cell body (soma), and the axon. Axon cells can respond to growth factors. The response is forwarded to the cell body, resulting in the expression of neuronal genes that make new proteins (Brittis, 2002).

Dendritic cells carry out the non-linear summation of input impulses, while the nuclear membrane in the cell body supports the processing of nerve signals persistently (Sidiropoulou *et al.*, 2006). In pyramidal neuron cells, the basal dendritic sum up the impulse signal as a sigmoid unit. Depolarisation occurs from a voltage increase from -65 millivolt (mV) to -55 mV. Na⁺ ions leave the cell through Na⁺ channels at a speed of 10⁸ ions per second. As a result, the voltage increases, and K⁺ channels are opened. K⁺ ions can enter the cell while Na⁺ cannot leave again because the channels are closed, causing hyperpolarisation (voltage drop from -65 mV to -75 mV) (Borisyyuk *et al.*, 2005). Depolarization and hyperpolarisation events along neuronal cells cause action potentials. The frequency of action potentials in the axon can be conducted up to 1000 times per second. Neuron cells adjust the pH so that action potentials can take place optimally. The extrinsic factors involved in pH adjustment are the neurotransmitter's amino acids or analogues, gamma-aminobutyric acid, glycine, and cyclic nucleotides. Astrocytes are the primary key to pH adjustments (Obara *et al.*, 2008).

Glia is much more than neurons in the human brain, including astrocytes, oligodendrocytes, and microglia. Astrocytes are one of the supporting cells in the central nervous system. Mihaly von Lenhossek proposed the name "astrocyte" in 1895. The name astrocyte was suggested because the shape of these cells resembles a star. In electron microscopic studies of astroglia, astrocytes have radially protruding filaments, glycogen granules, and an oval-shaped nucleus. Astrocyte cells in the brain can reach 20% of the total brain volume (Akdemir, 2020). Based on their morphological appearance, astrocyte cells are classified into protoplasmic and fibrous. Most protoplasmic types are deposited in the grey matter while fibrous in the white matter. Astrocytes have special membranes, namely gap junctions and orthogonal assemblies, which connect neuron cells and transport substances between astrocytes and blood (de la Rosa *et al.*, 1985). Astrocytes function to regulate K⁺ ions in the brain, synthesise neurotransmitters, reduce

neurotransmitters that induce pathophysiological conditions, and integrate metabolically with neurons (Weber & Barros, 2015). Astrocytes can express Glial Fibrillary Acidic Protein (GFAP) in SVZ. Astrocytes can be identified by staining immunohistochemistry on GFAP. Histologists usually use hematoxylin and eosin to visualise astrocytes (Wei & Morrison, 2023). Astrocytes are marked with a red appearance (Guillamón-Vivancos *et al.*, 2015). In addition, identifying astrocytes specific to certain types can see several biomarkers, such as connexin 30, Glutamate and Aspartate Transporter (GLAST)/Excitatory Amino Acid Transporter 1 (EAAT-1), Glutamate Transporter 1 (GLT-1)/EAAT-2, and kir4 potassium channels (Khakh & Sofroniew, 2015).

Microglia are small neuron cells that belong to glial cells. Microglia are deposited in the brain and spinal cord. Microglia can phagocytise pathogens that have entered the brain and are only active when there is an inflammatory process in the brain. It reached 5% of the brain. Microglia activation to phagocytise pathogens is influenced by astrocytes (Ovanesov *et al.*, 2008). Astrocytes help microglia by secreting Interleukin 1 (IL-1) and prostaglandins (Ludwig & Das, 2023). Microglia have scanty cytoplasm and are derived from differentiated monocytes. Microglia can increase the size of their nucleus. After that, the nucleus will stick to the necrotic tissue and form aggregates. The biomarker for microglia is filamentous vimentin and iba1 (Autilio-Gambetti *et al.*, 1982). Sustained nerve cell infection can initiate an increase in RANTES protein expression, which activates astrocyte cells. Activated astrocyte cells can secrete factors that activate microglial cells (Ovanesov *et al.*, 2008). Expression of Olig₂, Platelet-Derived Growth Factor Receptor Alfa (PDGFR α), and Neural Glial Antigen 2 (NG2) resulted in mature oligodendrocytes expressing Companion of Cellulose Synthase 1 (CC1), Glutathione S-Transferase Phi (GST- π), and myelin proteins in the adult brain using the NG2, PDGFR α , and Olig₂-CreER (Cre Estrogen Receptor) mouse lines.

Oligodendrocyte Progenitor Cells (OPC) are cells that will differentiate into CNS myelinating cells and oligodendrocytes. At the stage of neurogenesis, OPC is detected in the ventral CNS, which will develop and increase to all parts of the brain. OPC was first discovered by culturing cells. The detected OPCs were found in the spinal cord and forebrain. OPC cell surface can be found in several proteins, such as A2B5 antibody, NG2 proteoglycan, or PDGFR α receptor. OPC will proliferate and undergo mature development into myelin. With the proliferative activity of OPC, the protein on the OPC surface will disappear and will express several antigens, namely O4 monoclonal antibodies, galactocerebroside, 2',3'-cyclic nucleotide phosphodiesterase, and myelin essential protein.

Oligodendrocyte cells originate from the embryonic ventricles, spinal cord, and forebrain ventricles (Spassky *et al.*, 1998). The expression of several proteins in Sonic Hedgehog (SHH) and PDGFR α evidence this. In fact, at the spinal cord and forebrain sites, OPC produces two PDGF-responsive and non-PDGF-responsive cells. PDGF-responsive cells will develop into more OPCs and mature oligodendrocytes. The presence of Ascl1 also proves that OPC originating from the dorsal area will spread to the ventral region, such as the spinal cord.

Furthermore, neurons will experience deformation with age (Wyss-Coray, 2016). The process of neuron deformation is called neurodegenerative. One of the neurodegenerative signs due to ageing is DNA damage. Oligodendrocytes are more susceptible to DNA damage. DNA damage can be caused by exposure to Reactive Oxygen Species (ROS). Some sources of ROS are aged microglia, oligodendrocytes and APCs. The results of studies on Alzheimer's disease rats proved that removing aged cells can reduce neuroinflammation.

Infection in the brain can increase pro-inflammatory chemical compounds, such as TGF (Transforming Growth Factor)- β , IL-6, Leukaemia Inhibitory Factor (LIF), and Oncostatin M. These compounds can increase astrocyte activity in astrogliosis. Astrogliolysis causes inflammation in neuron cells continuously, causing neurodegeneration. Neurodegenerative diseases are Alzheimer's, Parkinson's, and multiple sclerosis due to neurodegeneration. One disease that is important to note is various sclerosis because it is a rare disease.

Multiple sclerosis

Symptoms and clinical course

Multiple sclerosis has no specific symptoms. The clinical manifestations of various sclerosis show almost all neurological symptoms, so they cannot be determined and predicted. Every patient shows different symptoms, and from time to time, clinical symptoms may change. Multiple sclerosis attacks almost all areas of the central nervous system due to its autoimmune nature. However, in some clinical symptoms that occur, some symptoms predominate (primary symptoms). The primary symptoms of multiple sclerosis are sensory disturbances, difficulty walking, problems with vision, and cognitive and emotional disorders (Gelfand, 2014).

Since 2013, multiple sclerosis has had three phenotypes: active disease, progressive disease or disease progressing, and worsening disease. Active disease is an acute/subacute episode of multiple sclerosis characterised by increasing neurologic dysfunction. It is also closely associated with

gadolinium-enhancing lesions. Active disease requires annual evaluation. Progression disease is an accrual disability that reaches the PPMS (Primary progressive multiple sclerosis) or SPMS (secondary progressive multiple sclerosis) phase.

Patients with relapsing-remitting multiple sclerosis (RRMS) status will experience SPMS if they do not receive therapy. In the progressive phase, dendritic cells, macrophages, and natural killer cells have a strategic role in the pathophysiology of multiple sclerosis. Changes in cytokines (IL-12 and IL-18) and costimulatory molecules in dendritic cells are signs of a change in status from RRMS to SPMS. RRMS pathology is associated with mitochondrial dysfunction and axonal damage. The pathology in PPMS is complex and includes neurodegeneration that occurs together with mild to moderate inflammation. Progression disease requires evaluation on an annual basis with clinical judgment. The worsening disease is an increase in disability due to increased progressive disability or post-recurrence residual deficit (Lublin *et al.*, 2020). The worsening disease does not require evaluation.

In addition, multiple sclerosis can be seen through several biomarkers. Some of the biomarkers are Cerebrospinal Fluid Immunoglobulin M Oligoclonal Bands (CSF IgM OCB), CSF C-X-C chemokine motif 13 (CXCL13), CSF chitinase-3-like-protein 1 (CH13L1), and CSF neurofilament light chain (NIL). The difference between PPMS and RRMS can be seen in the presence of microRNA, miR-223, and miR-15b serum. SPMS patients experienced decreased N-acetyl aspartate (NAA) levels, while RRMS patients did not. The presence of CSF-limited OCB IgM marked the phenotype change from RRMS to SPMS.

Etiology

Myelin is a network composed of protein-lipid-protein-lipid-protein. Lipid and protein components can be deformed, causing disease. The lipid and protein deformation process in myelin is called demyelination (Love, 2006). Some causes of demyelination are inflammation, viral infection, metabolic disorders, and local compression. Demyelination due to inflammatory agents causes plaque formation. Plaque is divided into active plaque, inactive plaque, chronic active plaque, and shadow plaque. Inflammatory agents (lymphocytes and macrophages) are abundantly deposited in active plaques. Recent research states that the leading cause of demyelination is unknown with certainty and comprehensively due to complex multifactorial disorders (Kamm *et al.*, 2014) (Solaro *et al.*, 2018). Myelin deformation can be further increased due to high levels of CD4+. In addition, external factors can also affect the development of demyelination, namely

exposure to viral infections, such as EBV, Human Herpes Virus 6 (HHV-6), vitamin D deficiency, and vitamin D deficiency.

Epidemiology

The prevalence of multiple sclerosis occurs in the subtropics. In central Europe and Hungary, the standard prevalence of multiple sclerosis for men and women is 53.9/100,000 and 144.8/100,000, respectively. Factors that led to an increase in the prevalence of multiple sclerosis in Hungary were a decrease in the number of deliveries, the severity of obesity, and an increase in tobacco consumption (Biernacki *et al.*, 2020). Epidemiological studies showcase that multiple sclerosis in Latin America and the Caribbean is 21.5 per 100,000 population. Levels of sun exposure cause differences in the prevalence of multiple sclerosis in Europe and Latin America, quality of drinking water and food, and vaccinations (Cristiano *et al.*, 2013). The incidence of multiple sclerosis is also found in East Asia, which is 0.8-2/100,000 population. China has a lower prevalence of MS than other Asian countries (Eskandarieh *et al.*, 2016). Epidemiological data show that the incidence of MS is increasing every year, even in areas of Asia where the incidence is low (Selewski *et al.*, 2010). The therapy currently used only relies on fingolimod, natalizumab, and alemtuzumab, so there is a need to discover new therapies, considering that the drugs currently used do not guarantee long-term effects. This is related to side effects, such as fingolimod (decreased lymphocyte levels), mitoxantrone (cardiotoxicity), and azathioprine (increased cancer risk) (Goudarzvand *et al.*, 2016).

Pathophysiology

Multiple sclerosis has several stages in its progression. The early progressive stage is characterised by primary demyelination and reactive gliosis. Subsequently, there is low-grade inflammation and microglia activation. Finally, the components of adaptive immunity will be active, causing ongoing demyelination and neurodegenerative disease (Huang *et al.*, 2017). Multiple sclerosis is closely related to autoimmune disease because CD8+ and microglia activation induce inflammation. The presence of CD8+ activation increases the concentration of neutrophils. An increase in neutrophil count is closely related to an increase in Toll-Like Receptor 2 (TLR2) concentration. It shows phenotypic changes for Formyl Peptide Receptor 1 (FPFR1), Chemokine Receptor Type 4 (CXCR), and Cluster of Differentiation 43 (CD43) expression. The NLR value will also increase (Meinl, 2006) (Naegele, 2012). NLR values correlate positively with increased multiple sclerosis disease (Wan, 2020). The deposited

CD8+ forms plaques in the white matter (brain stem, subpial in spinal cord, and cerebellar stem) and grey matter (Valori Id et al., 2021). Multiple sclerosis is closely related to Tumor Necrosis Factor-alpha (TNF-alpha) and Interferon-gamma (IFN-gamma). IFN-gamma at high concentrations increases Major Histocompatibility Complex (MHC) expression in oligodendrocytes (Horwitz, 1997). TNF-alpha can help macrophages infiltrate myelin cells. This is due to TNF-alpha signalling at the TNF P55 receptor (Akassoglou, 1998). In addition, mature oligodendrocytes play an active role in the remyelination process in multiple sclerosis. Oligodendrocyte cells are a differentiated form of NG2-Glia. NG2-glia are found in the dorsal subventricular zone, differentiating them from a single progenitor cell. NG2-Glia cells have phenotypic heterogeneity relative to their ontogenic origin. These cells are clonally distributed in white and grey matter (Wan, 2020). Granulocyte-macrophage colony-stimulating factor (GM-CSF) also plays a role in mediating neuroinflammation. GM-CSF triggers the accumulation of dendritic cells that present myelin antigens on CD4+. These cells will exclusively migrate to the CNS parenchyma and produce GM-CSF and IL-17. This causes a shift of Ly6C⁺CCR2⁺ cells from the bone marrow to the CNS. In the CNS, GM-CSF will modulate the differentiation of dendritic cells into monocytes and monocytes into macrophages. These cells can

release mediators that cause demyelination, tissue damage, and axonal loss (Monaghan, 2020).

The lesions in multiple sclerosis are carried out by inflammatory activity by T lymphocytes and macrophages with a distinct pattern. The pattern is based on the distribution of protein loss, plaque expansion, a pattern of oligodendrocyte destruction, and immunopathological evidence. Patterns I and II are distinguished by the deposition of IgS and the complement antigen C9neo. Pattern II occurs when there is active degeneration of the myelin margins. Pattern I and II lesions form demyelinated plaques centred on small veins and venules. Pattern III lesions do not form plaques in veins and venules. This lesion has no clear active lesion boundaries and has a diffuse spread to the white matter. Pattern IV lesions are associated with oligodendrocyte death in the periplaque white matter. This pattern lacks IgS deposition and C9neo complement antigen.

Neurotoxin-induced MS in animal model

Animal models for multiple sclerosis can be induced by toxic chemical compounds in the nervous system (neurotoxins), such as myelin essential protein (MBP), Myelin Oligodendrocyte Glycoprotein (MOG), Cuprizone, Lysolectin, Diphtheria toxin, and Ethidium bromide. Each neurotoxin has its mechanism for generating multiple sclerosis.

Table III: Neurotoxin-induced multiple sclerosis

Neurotoxin	Animal models	Dosage	Administration	Mechanism	Parameter	References
Myelin basic protein	Female lewis rate aged 8-12 weeks and guinea pig	MBP-specific T cells at a concentration of 3 × 10 ⁵ per milliliter were cultured for 2 days in RPMI 1640 Dutch modification medium supplemented with 1% homologous rat serum and additional components. During this incubation period, they were exposed to 10 µg per milliliter of MBP and 15 × 10 ⁶ per milliliter of irradiated syngeneic thymocytes	Intra Peritoneal	Stimulates CD4+ cells to demyelinate.	Kv1.3 channel and IKca1 channel	(Christine Beeton, 2001)
	zebrafish strains and lines: AB, Golden and Tg(sox10(7.2):mRFP)	1 nl of a solution containing 10 ng/µl plasmid DNA and 25 ng/µl tol2 transposase mRNA	Fertilised eggs were co-injected		Axon myelinated, the number of myelin sheath per oligodendrocytes, number of oligodendrocytes	(Almeida, 2011)
Myelin oligodendrocyte	C57BL/6 female rat (wild type, 18–	200 µg of synthetic myelin oligodendrocyte glycoprotein (MOG). The peptide was emulsified	Subcutaneous	Stimulates CD4+ cells to demyelinate	Loss of tail tone; partial hind limb paralysis; complete hind limb paralysis; complete	(Nathalia Bernardes Teixeira, 2020)

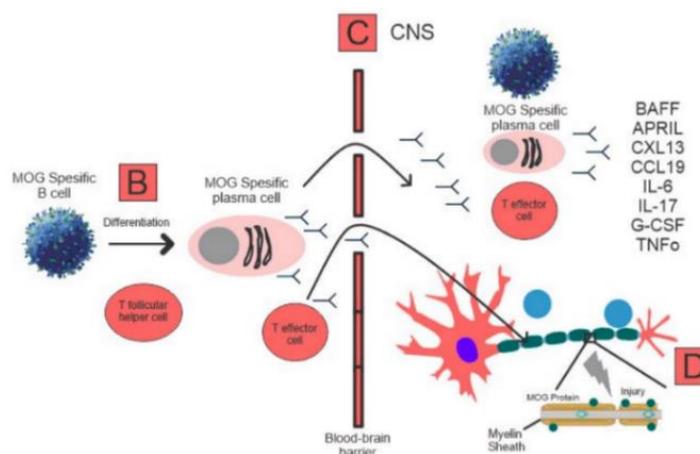
Neurotoxin	Animal models	Dosage	Administration	Mechanism	Parameter	References
glycoprotein (MOG) 35-55	22 g) aged 8–12 weeks	supplemented with 400 mg/mL of Mycobacterium tuberculosis and injected in a 200 µL volume.			paralysis of the hind limbs and part of the forelimbs; and decreased responsiveness (near death) and death (euthanasia)	
	Wild type zebrafish (Danio rerio)	MOG in Complete Freund's Adjuvant (CFA) 0.3 mg/mL; 0.6 mg/mL; 1 mg/mL	Subcutaneously (s.c.) in the mid spine regions		Clinical scores, body weight, and mortality	(Kulkarni, 2017)
	Monkeys	100 µg of MOG peptide dissolved in 300 µl of buffered saline and 300 µl of CFA	Injected into the dorsal skin		Clinical course, B cell response, T cell response, MRI of brains	(Yolanda S. Kap et al., 2008)
Cuprizone	Mice, rats, and guinea pig	0,5-2% (w/v) cuprizone	Oral	Disturbance of energy metabolism in oligodendroglia and cell function that leads to demyelination	Morphological assays of myelination and cerebroside counts	(Matsushima & Morell, 2001a)
	Wistar rats	0.6% cuprizone	Oral gavage		Nerve conductance velocity and histopathological tissue evaluation	(Basoglu et al., 2013)
	Larval zebrafish	40 µg/mL	Placing zebra fish larvae in a container containing cuprizone		Expression of myelin protein zero (MPZ), expression of inflammatory markers, expression of nitric oxide synthase	(Jaronen et al., 2022)
Lysolecithin	Twenty-four male mice of two strains (OLA/MF1 and ICI) were used in these experiments.	2 mL lysolecithin 1%	Injection into the spinal cord	Axonal lesions and demyelination occur	The length and location of lesions	(N. D. Jeffery & W. F. Blakemore, 1995)
	Male sparague-dawley rats	1% lysolecithin in normal saline	Injection to corpus callosum and straviuom on the right-hand side of the brain by stereostatic		C-11-Labeled N-methyl-4,4'-diaminostilbene, F-11-Labeled fludeoxyglucose	(de Paula Faria et al., 2014)
	Larval zebrafish	Lysolecithin was diluted to 0.875% concentration in saline from 1% stock concentration	Inserted into the larvae, starting from the dorsal spinal cord and moving ventrally within the spinal cord, between somites 16 and 18.		Myelinating glial cell loss, myelin perturbations, axonal sparing, and debris clearance.	(Morris & Kucenas, 2021)
Diphtheria toxin alfa (DTA)	MOG-Cre / IDTR mice	400 ng	Intraperitoneal	Inhibit protein synthesis	Number of damaged axons, activation of astrocytes and microglia, neuronal apoptosis, axon damage	(Ghosh et al., 2011a)
Diphtheria toxin	MBP-DTR transgenic mice	200 ng	Intraperitoneal	Inhibit protein synthesis	Number of tunnel cells, clinical (weak forelimbs, shortness of breath,	(Laura-Jane Oluich et al., 2012)

Neurotoxin	Animal models	Dosage	Administration	Mechanism	Parameter	References
receptors (DTR)					reduced mobility, and weight loss) and motor (handrails gripping after being grabbed by the tail, ataxic gait, tail spasticity, and thoracic kyphosis) deficits, electrophysiological abnormalities, number of oligodendrocyte cell bodies CC-1+	
Etidium bromide (EB)	Mice	2 µL of 0,05% (w/v) or 4 µL 0,1% (w/v)	Injection into the white matter tract	Intercalation of DNA	Histological analysis (immunochemistry, measurement of lesion area, assessment of the extent of remyelination, and axonal damage)	(Rachel H. Woodruff & Robin J.M. Franklin, 1999)
	Zebrafish	75µM	Zebrafish were distributed into 6-well plates that contain ethidium bromide		Motility assay, fluoromyelin staining, and whole mount anti-MBP immunostaining	(Zhu et al., 2019)
	Wistar rats	3 µL of 0.01% EB in sterile 0.9% saline	Injected into the right dentate gyrus of hippocampal		Behavioral evaluation (morris water maze and hidden platform experiments), ultrastructural of hippocampal formation)	(Goudarvand et al., 2016)

Discussion

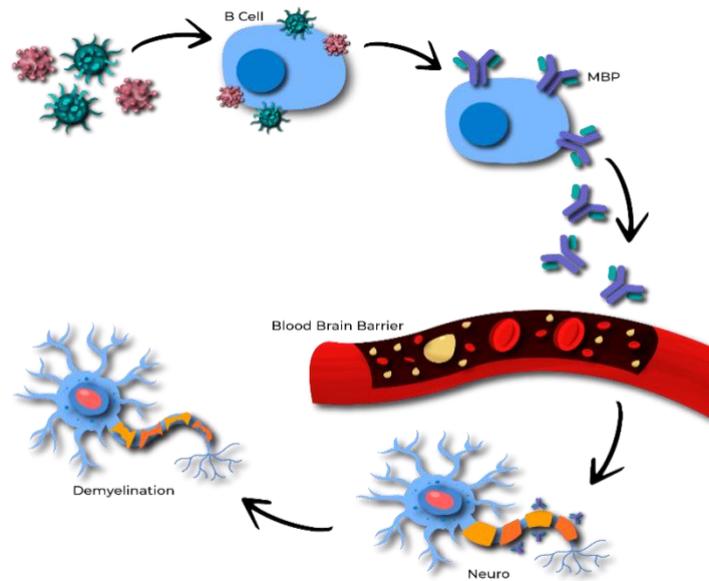
Neurotoxins are used to mimic the mechanism of multiple sclerosis. MOG and MBP have better survival rates. The use of MBP and MOG showed that all animal models were successful in surviving (Beeton *et al.*, 2001; Teixeira *et al.*, 2020). The combination of MBP and MOG in the manufacture of animal models has a rapid onset and high mortality rate (de Rosbo *et al.*,

1995). MOG and MBP are preferable to resemble MS pathophysiology in humans (Miyamura *et al.*, 2019). Therefore, these two neurotoxins are preferred for developing animal models to generate and mimic the pathogenesis of multiple sclerosis with secondary progressive forms compared to other neurotoxins (Tanabe *et al.*, 2019). Their molecular mechanism is described clearly in Figures 1 and 2.



Myelin oligodendrocytes will differentiate into MOG-specific plasma cells because the presence of helper T-cells influences them. T effector cells will infiltrate the CNS and induce B-cell Activating Factor (BAFF), A Proliferation-Inducing Ligand (APRIL), Collagen Cross-Linking (CXL) 13, CC Chemokine Ligand (CCL) 19, IL-6, IL-17, Granulocyte Colony-Stimulating Factor (G-CSF), and TNF-alpha, which cause axon demyelination.

Figure 1: Mechanism action of MOG-induced MS

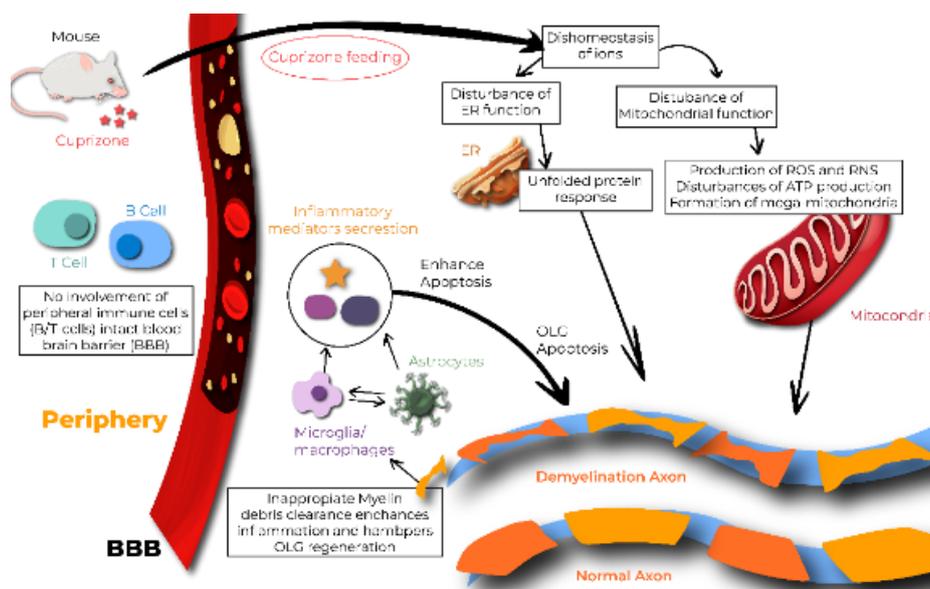


The presence of microbes with the MBP epitope causes this epitope to bind to B cells, which produce autoreactive B cells. Autoreactive B cells present the MBP antigen Abz. This antigen can penetrate the BBB (blood-brain barrier) and cause demyelination.

Figure 2: Mechanism of MBP-induced MS

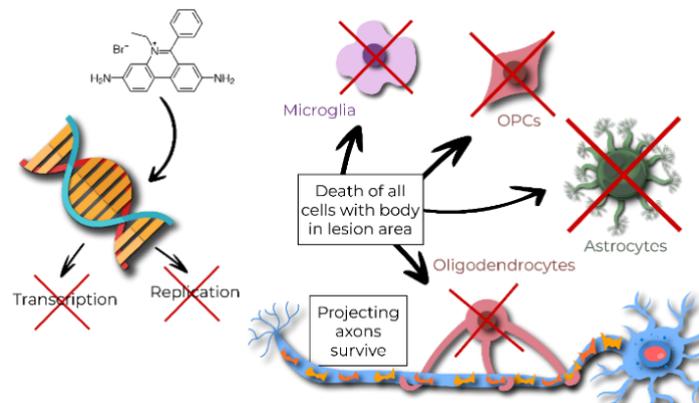
Instead, cuprizone is particularly relevant for use in studies addressing toxic mechanisms of the demyelination process and studies of therapeutic interventions (Figure 3). The mechanism of action of cuprizone represents lesions of patterns III and IV (Lucchinetti *et al.*, 2000). Ethidium bromide is commonly used for endogenous (Kuypers *et al.*, 2013).

Ethidium bromide causes demyelination in the spinal cord (Figure 4). A distinctive feature of ethidium bromide is that it causes the loss of oligodendrocytes and astrocytes. This observation indicates that ethidium bromide is less indicative of multiple sclerosis ((Matsushima & Morell, 2001b).



Cuprizone damages homeostasis in copper ions, thus initiating mitochondrial and endoplasmic reticulum dysfunction. This disruption results in ROS formation, impaired ATP formation, and an unfolded protein response. Oligodendrocytes will undergo apoptosis and form myelin debris. This myelin debris can mediate inflammatory media secretion that increases apoptosis in oligodendrocytes.

Figure 3: Mechanism of Cuprizone Induced MS

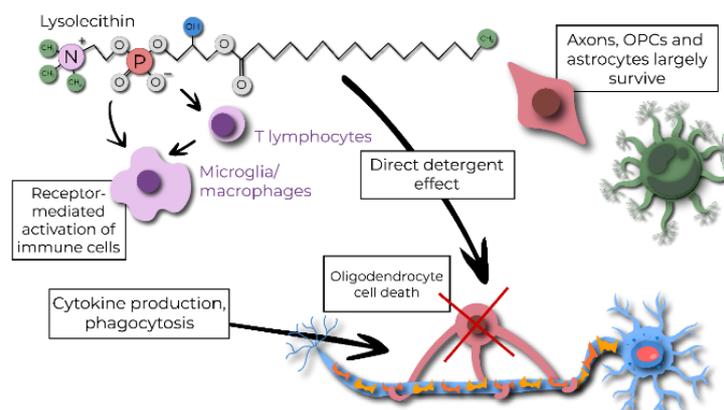


Ethidium bromide can intercalate DNA so that transcription and replication do not occur. It can also kill microglia nuclei, OPCs, astrocytes, and oligodendrocytes.

Figure 4: Mechanism of Cuprizone Induced MS

Furthermore, the chemical compound lysolecithin has a percentage of 90% to cause lesions in rats. Of the 20 rats treated with lysolecithin injection, 18 developed lesions due to lysolecithin exposure (Jeffery & Blakemore, 1995). Lysolecithin is suitable for old animal models because remyelination is relatively long. The

process of remyelination from lysolecithin exposure occurs within five to six weeks. The demyelination process of lysolecithin is well-controlled in animal models (74). The mechanism action of lysolecithin is described in Figure 5.

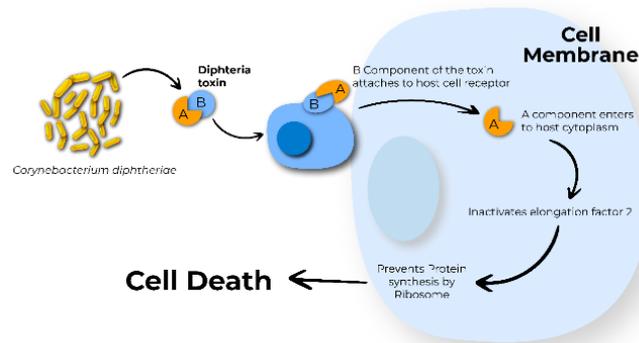


Lysolecithin can cause oligodendrocyte cell death through a direct detergent effect and activates immune cells.

Figure 5: Mechanism of Lysolecithin Induced MS

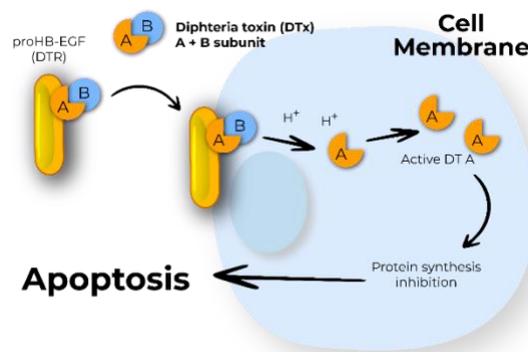
The MOGi-Cre/iDTR mouse model has not induced nerve cell death. This evidence shows that MOGi-Cre/iDTR mice are optimal for use as a model for multiple sclerosis because this disease only causes axonal injury (Ghosh *et al.*, 2011b). This axonal injury occurred over 230 days. In MOGi-Cre/iDTR mice, microglia activation was detected, similar to the pathophysiology of multiple sclerosis. The detailed mechanism is illustrated in Figure 6. However, massive neurotoxicity occurs in the MOGi-Cre/iDTR mouse

model, and the mechanism is not fully understood. MOGi-Cre/iDTR mice and MBP-DTR transgenic mice share the same clinical phenotype. MBP-DTR transgenic mice experienced a faster onset of clinical symptoms of multiple sclerosis, which occurred in 9-12 days. This mechanism is similar to diphtheria toxin or antigen (Figure 7). The limitations of the MBP-DTR transgenic mice are the absence of demyelination and loss of axons (Oluich *et al.*, 2012).



Diphtheria toxin has components A and B. Component B attaches to receptors on the host cell so that component A can go to the cell's cytoplasm. Component A inactivates elongation factor 2, which can prevent protein synthesis in cells and cause cell death.

Figure 6: Mechanism of DTA Induced MS



The mechanism for demyelination in the diphtheria toxin receptor is the same as that of the diphtheria toxin. However, proHB-EGF, which functions as a receptor, helps diphtheria toxin enter the cytoplasm.

Figure 7: Mechanism of DTR-induced MS

Several neurotoxins used in animal models of Alzheimer's can also be applied to animal models of multiple sclerosis because they share the exact mechanism. Scopolamine can increase malondialdehyde (MDA), nitrite dioxide (NO), and glutathione (GSH) so that they can cause oxidative stress (Somogyi *et al.*, 2021). In addition, scopolamine can increase proinflammatory cytokines, such as TNF-alpha, IL-1beta, and IL-18. Inflammatory processes occur in the hippocampus. The use of scopolamine as a test animal model has limitations in that higher doses of scopolamine can cause peripheral side effects that can affect the learning and memory abilities of the test animals. Colchicine can cause neuronal cell death through neuroinflammation (Sil & Ghosh, 2016). This inflammation exists because colchicine can increase the concentration of Cyclooxygenase (COX)-2. High COX-2 induces an increase in proinflammatory cytokines (TNF-alpha and IL-1beta). The increase in COX-2 appeared 21 days after the rats were given colchicine. L-methionine is also capable of causing neurodegeneration in CNS nerve cells after 12 weeks of

treatment (Wang *et al.*, 2020). This neurodegeneration is caused by neuroinflammation due to increased astrogliosis and microgliosis activity. In addition, an increase in GFAP can also occur after administration of L-methionine. However, no data shows this neurotoxin's application to animal models of multiple sclerosis testing, so further research is needed.

Conclusion

MOG and MBP are appropriate for generating animal multiple sclerosis models for further in vivo experiments.

Acknowledgement

The authors want to express our sincere gratitude to the Faculty of Military Pharmacy, Republic of Indonesia Defense University, the School of Pharmaceutical

Science, Universiti Sains Malaysia, and the Faculty of Pharmacy, Universitas Gadjah Mada, for their facilities, resources, and guidance which significantly contributed to the completion of this manuscript.

Author contributions

Conceptualisation: BW, NH; Methodology, Supervision: YP, BW, SS; Writing–Review and Editing: YP, BW, SS; Funding acquisition: BW, SS, NH.

Source of funding

The Faculty of Military Pharmacy Republic of Indonesia Defense University, School of Pharmaceutical Science Universiti Sains Malaysia, and Faculty of Pharmacy Universitas Gadjah Mada generously supported this research.

References

OpenStax College. (n.d). 7.1: *Anatomy of the nervous system*. Biology LibreTexts. Retrieved July 11, 2023, from [https://bio.libretexts.org/Courses/Lumen_Learning/Biology_of_Aging_\(Lumen\)/07%3A_The_Nervous_System/7.01%3A_Anatomy_of_the_Nervous_System](https://bio.libretexts.org/Courses/Lumen_Learning/Biology_of_Aging_(Lumen)/07%3A_The_Nervous_System/7.01%3A_Anatomy_of_the_Nervous_System)

Adams, M. E., & Olivera, B. M. (1994). Neurotoxins: overview of an emerging research technology. *Trends in Neurosciences*, **17**(4), 151–155. [https://doi.org/10.1016/0166-2236\(94\)90092-2](https://doi.org/10.1016/0166-2236(94)90092-2)

Akassoglou, K., B. J., K. G., P. M., L. H., K. G., & P. L. (1998). Oligodendrocyte apoptosis and primary demyelination induced by local TNF/p55TNF receptor signaling in the central nervous system of transgenic mice: Models for multiple sclerosis with primary oligodendroglial pathology. *The American Journal of Pathology*, **153**(3), 801–813.

Akdemir, E. S., H. A. Y., & D. B. (2020). Astrocytogenesis: where, when, and how. *F1000Research*, **9**.

Almeida, R. G., C. T., F.-C. C., & L. D. A. (2011). Individual axons regulate the myelinating potential of single oligodendrocytes in vivo. *Development (Cambridge, England)*, **138**(20), 4443–4450.

American Association of Neurological Surgeons. (2023). *Anatomy of the Brain*. <https://www.aans.org/en/Patients/Neurosurgical-Conditions-and-Treatments/Anatomy-of-the-Brain>.

Autilio-Gambetti, L., Sipple, J., Sudilovsky, O., & Gambetti, P. (1982). Intermediate filaments of Schwann cells. *Journal of Neurochemistry*, **38**(3), 774–780. <https://doi.org/10.1111/J.1471-4159.1982.TB08698.X>

Basoglu, H. A. R. U. N., Boylu, N. T., & KOSE, H. (2013). Cuprizone-induced demyelination in Wistar rats;

electrophysiological and histological assessment. *European Review for Medical & Pharmacological Sciences*, **17**(20).

Beeton, C., Wulff, H., Barbara, J., Clot-Faybess, O., Pennington, M., Bernard, D., Cahalan, M. D., Chandy, K. G., & Béraud, E. (2001). Selective blockade of T lymphocyte K⁺ channels ameliorates experimental autoimmune encephalomyelitis, a model for multiple sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*, **98**(24), 13942. <https://doi.org/10.1073/PNAS.241497298>

Biernacki, T., Sandi, D., Friczka-Nagy, Z., Kincses, Z. T., Füvesi, J., Laczkó, R., Kokas, Z., Klivényi, P., Vécsei, L., & Bencsik, K. (2020). Epidemiology of multiple sclerosis in Central Europe, update from Hungary. *Brain and Behavior*, **10**(5). <https://doi.org/10.1002/BRB3.1598>

Borisjuk, A., Friedman, A., Ermentrout, B., & Terman, D. (2005). *Tutorials in Mathematical Biosciences I. 1860*. <https://doi.org/10.1007/B102786>

Brittis, P. A., L. Q., & F. J. G. (2002). Axonal protein synthesis provides a mechanism for localized regulation at an intermediate target. *Cell*, **2**(110), 223–235.

Carota, A., Calabrese, P., & Bogousslavsky, J. (2016). Neurotoxic Weapons and Syndromes. *Frontiers of Neurology and Neuroscience*, **38**, 214–227. <https://doi.org/10.1159/000442658>

Chayer, C., & Freedman, M. (2001). Frontal lobe functions. *Current Neurology and Neuroscience Reports*, **1**(6), 547–552. <https://doi.org/10.1007/S11910-001-0060-4>

Christine Beeton, H. W. J. B. O. C.-F. M. P. D. B. M. D. C. K. G. C. and E. B. (2001). Selective blockade of T lymphocyte K⁺ channels ameliorates experimental autoimmune encephalomyelitis, a model for multiple sclerosis. *Proceedings of the National Academy of Sciences*, **98**(24), 13942–13947.

Cristiano, E., Rojas, J. I., Romano, M., Frider, N., MacHnicki, G., Giunta, D. H., Calegario, D., Corona, T., Flores, J., Gracia, F., Maclas-Islas, M., & Correale, J. (2013). The epidemiology of multiple sclerosis in Latin America and the Caribbean: a systematic review. *Multiple Sclerosis (Houndmills, Basingstoke, England)*, **19**(7), 844–854. <https://doi.org/10.1177/1352458512462918>

de la Rosa, C., Cano, J., & Reinoso-suárez, F. (1985). An electron microscopic study of astroglia and oligodendroglia in the lateral geniculate nucleus of aged rats. *Mechanisms of Ageing and Development*, **29**(3), 267–281. [https://doi.org/10.1016/0047-6374\(85\)90067-3](https://doi.org/10.1016/0047-6374(85)90067-3)

de Paula Faria, D., de Vries, E. F., Sijbesma, J. W., Buchpiguel, C. A., Dierckx, R. A., & Copray, S. C. (2014). PET imaging of glucose metabolism, neuroinflammation and demyelination in the lysolecithin rat model for multiple sclerosis. *Multiple Sclerosis Journal*, **20**(11), 1443–1452.

de Rosbo, N. K., Mendel, I., & Ben-Nun, A. (1995). Chronic relapsing experimental autoimmune encephalomyelitis with a delayed onset and an atypical clinical course, induced in PL/J mice by myelin oligodendrocyte glycoprotein (MOG)-derived peptide: Preliminary analysis of MOG T cell epitopes. *European Journal of Immunology*, **25**(4), 985–993. <https://doi.org/10.1002/eji.1830250419>

- Eskandarieh, S., Heydarpour, P., Minagar, A., Pourmand, S., & Sahraian, M. A. (2016). Multiple sclerosis epidemiology in East Asia, South East Asia and South Asia: A systematic review. *Neuroepidemiology*, **46**(3), 209–221. <https://doi.org/10.1159/000444019>
- ET, O. D. C. (2007). *Understanding the Brain: The Birth of a Learning Science*. Centre for Educational Research and Innovation.
- Gautam, A. (2017). Nerve cells. *Encyclopedia of Animal Cognition and Behavior*, 1–3. https://doi.org/10.1007/978-3-319-47829-6_1282-1
- Gelfand, J. M. (2014). Multiple sclerosis: Diagnosis, differential diagnosis, and clinical presentation. *Handbook of Clinical Neurology*, **122**, 269–290. <https://doi.org/10.1016/B978-0-444-52001-2.00011-X>
- Ghosh, A., Manrique-Hoyos, N., Voigt, A., Schulz, J. B., Kreutzfeldt, M., Merkler, D., & Simons, M. (2011a). Targeted ablation of oligodendrocytes triggers axonal damage. *PLoS One*, **6**(7).
- Ghosh, A., Manrique-Hoyos, N., Voigt, A., Schulz, J. B., Kreutzfeldt, M., Merkler, D., & Simons, M. (2011b). Targeted ablation of oligodendrocytes triggers axonal damage. *PLOS ONE*, **6**(7), e22735. <https://doi.org/10.1371/JOURNAL.PONE.0022735>
- Goodkin, D. E. (1996). Interferon beta treatment for multiple sclerosis: Persisting questions. <http://Dx.Doi.Org/10.1177/135245859600100605>, **1**(6), 321–324. <https://doi.org/10.1177/135245859600100605>
- Goudarzvand, M., Choopani, S., Shams, A., Javan, M., Khodaii, Z., Ghamsari, F., Naghdi, N., Piryaei, A., & Haghparast, A. (2016). Focal injection of ethidium bromide as a simple model to study cognitive deficit and its improvement. *Basic and Clinical Neuroscience*, **7**(1), 63. <http://www.ncbi.nlm.nih.gov/pmc/articles/pmc4892333/>
- Guillamón-Vivancos, T., Gómez-Pinedo, U., & Matías-Guiu, J. (2015). Astrocytes in neurodegenerative diseases (I): function and molecular description. *Neurologia (Barcelona, Spain)*, **30**(2), 119–129. <https://doi.org/10.1016/J.NRL.2012.12.007>
- Herculano-Houzel, S. (2012). Neuronal scaling rules for primate brains. The primate advantage. *Progress in Brain Research*, **195**, 325–340. <https://doi.org/10.1016/B978-0-444-53860-4.00015-5>
- Hofman, M. A. (2014). Evolution of the human brain: When bigger is better. *Frontiers in Neuroanatomy*, **8**(MAR), 78485. <https://doi.org/10.3389/FNANA.2014.00015/BIBTEX>
- Horwitz, M. S., E. C. F., M. D. B., R. M., & O. M. B. (1997). Primary demyelination in transgenic mice expressing interferon- γ . *Nature Medicine*, **3**(9), 1037–1041.
- Huang, W. J., Chen, W. W., & Zhang, X. (2017). Multiple sclerosis: Pathology, diagnosis and treatments. *Experimental and Therapeutic Medicine*, **13**(6), 3163. <https://doi.org/10.3892/ETM.2017.4410>
- Jaronen, M., W., M. A., & Quintana, F. J. (2022). Protocol for inducing inflammation and acute myelin degeneration in larval zebrafish. *STAR Protocols*, **3**(1).
- Jeffery, N. D., & Blakemore, W. F. (1995). Remyelination of mouse spinal cord axons demyelinated by local injection of lysolecithin. *Journal of Neurocytology*, **24**(10), 775–781. <https://doi.org/10.1007/BF01191213>
- Juan Segura-Aguilar. (2001). *Mechanisms of degeneration and protection of the dopaminergic system* (First Edition).
- Kamm, C. P., Uitdehaag, B. M., & Polman, C. H. (2014). Multiple sclerosis: Current knowledge and future outlook. *European Neurology*, **72**(3–4), 132–141. <https://doi.org/10.1159/000360528>
- Khakh, B. S., & Sofroniew, M. V. (2015). Diversity of astrocyte functions and phenotypes in neural circuits. *Nature Neuroscience*, **18**(7), 942–952. <https://doi.org/10.1038/NN.4043>
- Kostrzewa, R. M. (2009). Neurotoxins. *Encyclopedia of Neuroscience*, 1035–1041. <https://doi.org/10.1016/B978-008045046-9.00519-2>
- Kretschmar, B., Pellkofer, H., & Weber, M. S. (2016). The use of oral disease-modifying therapies in multiple sclerosis. *Current Neurology and Neuroscience Reports*, **16**(4). <https://doi.org/10.1007/S11910-016-0639-4>
- Kulkarni, P., Y. S., M. R., S. D., S. U., & Y. Pn. zebrafish E. model: A quick in vivo screen for multiple sclerosis. (2017). Novel zebrafish EAE model: A quick in vivo screen for multiple sclerosis. *Multiple Sclerosis and Related Disorders*, **11**, 32–39.
- Kuypers, N. J., James, K. T., Enzmann, G. U., Magnuson, D. S. K., & Whittemore, S. R. (2013). Functional consequences of ethidium bromide demyelination of the mouse ventral spinal cord. *Experimental Neurology*, **247**, 615. <https://doi.org/10.1016/J.EXPNEUROL.2013.02.014>
- Laura-Jane Oluich, Jo Anne S. Stratton, Yao Lulu Xing, Sze Woei Ng, Holly S. Cate, Pankaj Sah, François Windels, Trevor J. Kilpatrick, & Tobias D. Merson. (2012). Targeted Ablation of Oligodendrocytes Induces Axonal Pathology Independent of Overt Demyelination. *Journal of Neuroscience*, **32**(24), 8317–8330.
- Love, S. (2006). Demyelinating diseases. *Journal of Clinical Pathology*, **59**(11), 1151–1159. <https://doi.org/10.1136/JCP.2005.031195>
- Lu, Q. R., Sun, T., Zhu, Z., Ma, N., Garcia, M., Stiles, C. D., & Rowitch, D. H. (2002). Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. *Cell*, **109**(1), 75–86. [https://doi.org/10.1016/S0092-8674\(02\)00678-5](https://doi.org/10.1016/S0092-8674(02)00678-5)
- Lublin, F. D., Coetzee, T., Cohen, J. A., Marrie, R. A., & Thompson, A. J. (2020). The 2013 clinical course descriptors for multiple sclerosis: A clarification. *Neurology*, **94**(24), 1088–1092. <https://doi.org/10.1212/WNL.0000000000009636>
- Lucchinetti, C., Brück, W., Parisi, J., Scheithauer, B., Rodriguez, M., Lassmann, H., & Heterogeneity, L. H. (2000).

Heterogeneity of multiple sclerosis lesions: Implications for the pathogenesis of demyelination.

Ludwig, P. E., & Das, J. M. (2023). Histology, glial cells. *StatPearls*.
<https://www.ncbi.nlm.nih.gov/books/NBK441945/>

Matsushima, G. K., & Morell, P. (2001a). The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. *Brain Pathology*, **11**(1), 107–116.

Matsushima, G. K., & Morell, P. (2001b). The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. *Brain Pathology*, **11**(1), 107–116. <https://doi.org/10.1111/J.1750-3639.2001.TB00385.X>

McLachlan, N. M., & W. S. J. (2017). The contribution of brainstem and cerebellar pathways to auditory recognition. *Frontiers in Psychology*, **8**(265).

Meinl, E., K. M., & H. R. (2006). B lineage cells in the inflammatory central nervous system environment: Migration, maintenance, local antibody production, and therapeutic modulation. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, **59**(6), 880–892.

Ming, G. Li, & Song, H. (2011). Adult neurogenesis in the mammalian brain: Significant answers and significant Questions. *Neuron*, **70**(4), 687.
<https://doi.org/10.1016/J.NEURON.2011.05.001>

Miyamura, S., Matsuo, N., Nagayasu, K., Shirakawa, H., & Kaneko, S. (2019). Myelin Oligodendrocyte Glycoprotein 35-55 (MOG 35-55)-induced experimental autoimmune encephalomyelitis: A model of chronic multiple sclerosis. *Bio-Protocol*, **9**(24).
<https://doi.org/10.21769/BIOPROT.3453>

Monaghan, K. L., & W. E. C. (2020). The role of granulocyte-macrophage colony-stimulating factor in murine models of multiple sclerosis. *Cells*, **9**(3), 611.

Morris, A. D., & Kucenas, S. (2021). A novel lysolecithin model for visualizing damage in vivo in the larval zebrafish spinal cord. *Frontiers in Cell and Developmental Biology*, **9**.

N. D. Jeffery, & W. F. Blakemore. (1995). *Remyelination of mouse spinal cord axons demyelinated by local injection of lysolecithin*. **24**(10), 775–781.

Nathalia Bernardes Teixeira, G. P. A. C. G. F. B. G. P. B. K. D. S. E. B. (2020). Alterations of peripheral nerve excitability in an experimental autoimmune encephalomyelitis mouse model for multiple sclerosis. *Journal of Neuroinflammation*, **17**(1), 1–14.

Obara, M., Szeliga, M., & Albrecht, J. (2008). Regulation of pH in the mammalian central nervous system under normal and pathological conditions: Facts and hypotheses. *Neurochemistry International*, **52**(6), 905–919.
<https://doi.org/10.1016/J.NEUINT.2007.10.015>

Olivia Guy-Evans. (2023, September 22). *An easy guide to neuron anatomy with diagrams*.
<https://www.simplypsychology.org/Neuron.html>.

Oluich, L.-J., Stratton, J. A. S., Xing, Y. L., Ng, S. W., Cate, H. S., Sah, P., Windels, F., Kilpatrick, T. J., & Merson, T. D. (2012). *Neurobiology of disease targeted ablation of oligodendrocytes induces axonal pathology independent of overt demyelination*.
<https://doi.org/10.1523/JNEUROSCI.1053-12.2012>

Ovanesov, M. V., Ayhan, Y., Wolbert, C., Moldovan, K., Sauder, C., & Pletnikov, M. V. (2008a). Astrocytes play a key role in activation of microglia by persistent Borna disease virus infection. *Journal of Neuroinflammation*, **5**, 50.
<https://doi.org/10.1186/1742-2094-5-50>

Ovanesov, M. V., Ayhan, Y., Wolbert, C., Moldovan, K., Sauder, C., & Pletnikov, M. V. (2008b). *Astrocytes play a key role in activation of microglia by persistent Borna disease virus infection*. <https://doi.org/10.1186/1742-2094-5-50>

Rachel H. Woodruff, & Robin J.M. Franklin. (1999). *Demyelination and remyelination of the caudal cerebellar peduncle of adult rats following stereotaxic injections of lysolecithin, ethidium bromide, and complement/anti-galactocerebroside: A comparative study*. **25**(3), 216–228.

Selewski, D. T., Shah, G. V., Segal, B. M., Rajdev, P. A., & Mukherji, S. K. (2010a). Natalizumab (Tysabri). *AJNR: American Journal of Neuroradiology*, **31**(9), 1588.
<https://doi.org/10.3174/AJNR.A2226>

Selewski, D. T., Shah, G. V., Segal, B. M., Rajdev, P. A., & Mukherji, S. K. (2010b). Natalizumab (Tysabri). In *American Journal of Neuroradiology* (Vol. 31, Issue 9, pp. 1588–1590).
<https://doi.org/10.3174/ajnr.A2226>

Serlin, Y., Shelef, I., Knyazer, B., & Friedman, A. (2015). Anatomy and physiology of the blood-brain barrier. *Seminars in Cell & Developmental Biology*, **38**, 2–6.
<https://doi.org/10.1016/J.SEMCDB.2015.01.002>

Shackelford, T., & V. J. (2017). *Encyclopedia of animal cognition and behavior*.

Sidiropoulou, K., Pissadaki, E. K., & Poirazi, P. (2006). Inside the brain of a neuron. *EMBO Reports*, **7**(9), 886.
<https://doi.org/10.1038/SJ.EMBOR.7400789>

Sil, S., & Ghosh, T. (2016). Role of cox-2 mediated neuroinflammation on the neurodegeneration and cognitive impairments in colchicine induced rat model of Alzheimer's Disease. *Journal of Neuroimmunology*, **291**, 115–124.
<https://doi.org/10.1016/J.JNEUROIM.2015.12.003>

Solaro, C., Gamberini, G., & Masuccio, F. G. (2018). Depression in Multiple Sclerosis: Epidemiology, Aetiology, Diagnosis and Treatment. *CNS Drugs*, **32**(2), 117–133.
<https://doi.org/10.1007/S40263-018-0489-5>

Somogyi, G., Hlatky, D., Spisák, T., Spisák, Z., Nyitrai, G., & Czurkó, A. (2021). Deciphering the scopolamine challenge rat model by preclinical functional MRI. *Scientific Reports*, **11**(1), 1–12. <https://doi.org/10.1038/s41598-021-90273-9>

Spassky, N., Goujet-Zalc, C., Parmantier, E., Olivier, C., Martinez, S., Ivanova, A., Ikenaka, K., Macklin, W., Cerruti, I., Zalc, B., & Thomas, J. L. (1998). Multiple restricted origin of oligodendrocytes. *The Journal of Neuroscience*, **18**(20), 8331. <https://doi.org/10.1523/JNEUROSCI.18-20-08331.1998>

- Spencer, P. S., & Lein, P. J. (2014). Neurotoxicity. *Encyclopedia of Toxicology: Third Edition*, 489–500. <https://doi.org/10.1016/B978-0-12-386454-3.00169-X>
- Tanabe, S., Saitoh, S., Miyajima, H., Itokazu, T., & Yamashita, T. (2019). Microglia suppress the secondary progression of autoimmune encephalomyelitis. *Glia*, **67**(9), 1694–1704. <https://doi.org/10.1002/GLIA.23640>
- Taylor, B., Hardcastle, C., & Marsiske, M. (2019). Central nervous system. *Encyclopedia of Gerontology and Population Aging*, 1–4. https://doi.org/10.1007/978-3-319-69892-2_668-1
- Teixeira, N. B., Picolo, G., Giardini, A. C., Boumezbeur, F., Pottier, G., Kuhnast, B., Servent, D., & Benoit, E. (2020). Alterations of peripheral nerve excitability in an experimental autoimmune encephalomyelitis mouse model for multiple sclerosis. *Journal of Neuroinflammation*, **17**(1), 1–14. <https://doi.org/10.1186/S12974-020-01936-9/FIGURES/10>
- Thau, L., Reddy, V., & Singh, P. (2022). Anatomy, central nervous system. *BMJ*, **1**(4293), 478–478. <https://doi.org/10.1136/bmj.1.4293.478>
- Torday, J. S., & Miller, W. B. (2016). On the evolution of the mammalian brain. *Frontiers in Systems Neuroscience*, **10**(APR), 181440. <https://doi.org/10.3389/FNSYS.2016.00031/BIBTEX>
- Tselis, A., Khan, O., & Lisak, R. P. (2007). Glatiramer acetate in the treatment of multiple sclerosis. *Neuropsychiatric Disease and Treatment*, **3**(2), 259. <https://doi.org/10.2147/NEDT.2007.3.2.259>
- Valori Id, M., Jansson, L., & Tienari, P. J. (2021). *CD8+ cell somatic mutations in multiple sclerosis patients and controls-Enrichment of mutations in STAT3 and other genes implicated in hematological malignancies*. <https://doi.org/10.1371/journal.pone.0261002>
- Wan, E. C. (2020). Cellular and Molecular Mechanisms in the Pathogenesis of Multiple Sclerosis. *Cells*, **9**(10), 2223.
- Wang, P., Wang, Y., Zhang, Q., Zhang, H., Li, Z., Liu, X., Kaur, L., & Kumar, M. (2020). Amelioration of cognitive deficits by *Spirulina platensis* in L-methionine-induced rat model of vascular dementia. *Pharmacognosy Magazine*, **16**(68), 133. https://doi.org/10.4103/pm.pm_438_19
- Weber, B., & Barros, L. F. (2015). The Astrocyte: Powerhouse and recycling center. *Cold Spring Harbor Perspectives in Biology*, **7**(12), 20396–20397. <https://doi.org/10.1101/CSHPERSPECT.A020396>
- Wei, D. C., & Morrison, E. H. (2023). Histology, astrocytes. *StatPearls*. <https://www.ncbi.nlm.nih.gov/books/NBK545142/>
- Wyss-Coray, T. (2016). *Ageing, neurodegeneration and brain rejuvenation HHS Public Access*. <https://doi.org/10.1038/nature20411>
- Yolanda S. Kap, Paul Smith, S. Anwar Jagessar, Ed Remarque, Erwin Blezer, Gustav J. Strijkers, Jon D. Laman, Rogier Q., Hintzen, Jan Bauer, Herbert P. M. Brok, & Bert A. 't Hart. (2008). Fast progression of recombinant human Myelin/Oligodendrocyte Glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis in marmosets is associated with the activation of MOG34–56-specific cytotoxic T cells. *The Journal of Immunology*, **180**(3), 1326–1337.
- Zhu, X. Y., Guo, S. Y., Xia, B., Li, C. Q., Wang, L., & Wang, Y. H. (2019). Development of zebrafish demyelination model for evaluation of remyelination compounds and RORyt inhibitors. *Journal of Pharmacological and Toxicological Methods*, **98**.