International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Dihydroorotate Dehydrogenase Inhibitors as Emerging Therapeutic Targets in Multiple Sclerosis: Mechanistic Insights and Therapeutic Perspectives

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Abstract

Multiple sclerosis (MS) is a chronic autoimmune and neurodegenerative disorder that associated with neuroinflammation, demyelination, and progressive axonal damage within the central nervous system (CNS). There occurs a complex interplay between genetic susceptibility, environmental triggers, and immune dysregulation, leading to the activation of autoreactive lymphocytes that target myelin antigen components which is the basic etiology behind the progression of the disease. There exists a number of limitations for the current available disease-modifying therapies of Multiple sclerosis such as partial efficacy, adverse effects, and the inability to completely cease progression of the disease. Recent advances in molecular immunology and the emerging evidences have demonstrated certain metabolic pathways as essential promising therapeutic targets involved in immune cell proliferation. A mitochondrial enzyme, Dihydroorotate dehydrogenase (DHODH is one e among the critical advanced target which is responsible for the rate-limiting step in de novo pyrimidine synthesis, and plays a crucial role in the proliferation of activated T and B lymphocytes. Restraining DHODH selectively suppresses actively dividing immune cells without significantly affecting resting immune cells, thereby contributing a targeted immunomodulatory approach. Oral disease modifying Drugs such as teriflunomide have already demonstrated clinical efficacy in relapsing forms of MS, while next generation DHODH inhibitors including vidofludimus (IMU-838) are currently being explored for added benefits like selectivity, safety and potency. This review delineates the recent understandings of the epidemiology and pathophysiology of MS, underlines the molecular mechanisms responsible for immune-mediated neurodegeneration, and explore the promising therapeutic significance of DHODH inhibitors. In addition to that, This review explores novel brakthrough, potential biomarkers, and future investigational concepts for therapies that targeting DHODH. Additionally, it discusses the role of DHODH inhibition in the development of next-generation treatment strategies for multiple sclerosis.

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune disorder that chiefly affects the brain and spinal cord and is characterized by neuroinflammation, demyelination, and progressive neurodegeneration.[1] The disease leads to the development of inflammatory lesions or plaques that impair the normal neuronal impulse transmission in the central nervous system.[2] The different clinical forms of MS includes **Clinically Isolated Syndrome (CIS), Relapsing-Remitting MS (RRMS), Secondary Progressive MS (SPMS),**

and Primary Progressive MS (PPMS). Among these, RRMS accounts for approximately 85% of diagnosed cases.

Although both developing and developed countries shows similar prevalence of MS, here exists a distinguished geographical pattern for the prevalence of MS. Regions near to the equator shown lower incidence rates reported. [2] Even though, Environmental factors such as sunlight exposure, vitamin D levels, and lifestyle differences have been suggested to contribute to this variation, the occurrence of MS is independent of age, sex and genetics. Epidemiological studies indicate a steady global rise in MS prevalence, with an estimated increase of approximately **0.6 million cases between 2013 and 2023**.

The clinical signs and symptoms of multiple sclerosis (MS) can vary greatly, including cognitive impairment, limb weakness, difficulty coordinating voluntary movements, visual impairment, sadness, and anxiety. [3]. Although B lymphocytes and innate immune cells play a significant role, T cells are primarily responsible for the autoimmune responses that drive the progression of multiple sclerosis.

During MS diagnosis, numerous clinical and paraclinical factors are taken into consideration. Between 2017 and 2024, the McDonald criteria received multiple modifications to improve the sensitivity and specificity of the diagnosis process and decrease misdiagnosis problems. [5].

The therapeutic approaches ranging from moderate efficacy medication therapy to intermediate efficacy medication therapy. The base of all the treatment strategies is prognostic criteria. One of the novel therapeutic options for patients with aggressive illness and are not responsive to conventional care is Autologous hematopoietic stem cell transplantation (AHSCT). An oral immunomodulator used once day for relapsing MS is Teriflunomide, one of the current therapy approaches. It exhibits prominent anti-inflammatory activity and decreases the lymphocytes activation which is the prominent process in multiple sclerosis. Teriflunomide belongs to the class of drugs known as dihydroorotate dehydrogenase (DHODH) inhibitors, which are currently being presented as new treatments for multiple sclerosis. DHODH is a novel component which is demonstrated as a crucial moiety for immune responses and the most unrewarded therapeutic target for multiple sclerosis.[14].

DHODH inhibitors can limit de novo pyrimidine synthesis thereby selectively decrease pathogenic immunological responses of multiple sclerosis (MS) while maintaining normal immune function. The molecular groundworks of MS pathogenesis are accounted in this review, which also illustrates the therapeutic potential of DHODH inhibitors in the treatment of the chronic autoimmune demyelinating illness.[16].

2. Epidemiology and Risk Factors of Multiple Sclerosis

Regional variation is one of the major factors affects the prevalence of MS, with temperate regions reporting far greater incidence and prevalence rates than locations close to the equator. This points out the link between MS prevalence and exposure to sunshine and vitamin D production. Locations at high latitudes produce less vitamin D due to less UV light, which has been linked to immunological imbalances and rised vulnerability to autoimmune diseases like multiple sclerosis. It has been revealed that MS is multifaceted, occurs from a complicated interplay between environmental factors and genetic predisposition. The disease susceptibility and development is influenced by numerous risk variables among which genetic susceptibility plays a major role, evidently, immune related genes such as human leukocyte antigen (HLA) undergo polymorphisms in MS pathogenesis. To add to it, MS shows a strong sex bias, with the highest prevalence rate in females which is approximately two to three times more than males, linking a significance of hormonal and immunological differences in disease pathogenesis.

Lifestyle factors and metabolic regulations also contribute to disease risk; particularly, increased likelihood in the MS progression in adolescent obesity later in life, associatind due to chronic low-grade inflammation and altered immune regulation. Limited sunexposure, thereby decreased vitamin D production have also been illustrated as environmental factors that greatly correlated with increased MS risk and disease activity. Another important correlating factor are Infectious agents, for instance, viral infections such as Epstein–Barr virus (EBV), which has been consistently linked with MS onset and is considered one of the strongest environmental risk factors for the disease. [24]

There has been a bunch of discussions related with multifactorial influences on MS risk and prevalences such as cytomegalovirus (CMV) infection on immune system, exposure to contaminants in the environment and high altitudes. Ultimately the immune regulations are negatively influenced by these genetic, environmental, and lifestyle factors and may leads to autoimmune reactions against oligodendrocyte proliferation thereby, myelin components of the central nervous system eventually leading to the onset and advancement of multiple sclerosis.

3. Pathophysiology of Multiple Sclerosis

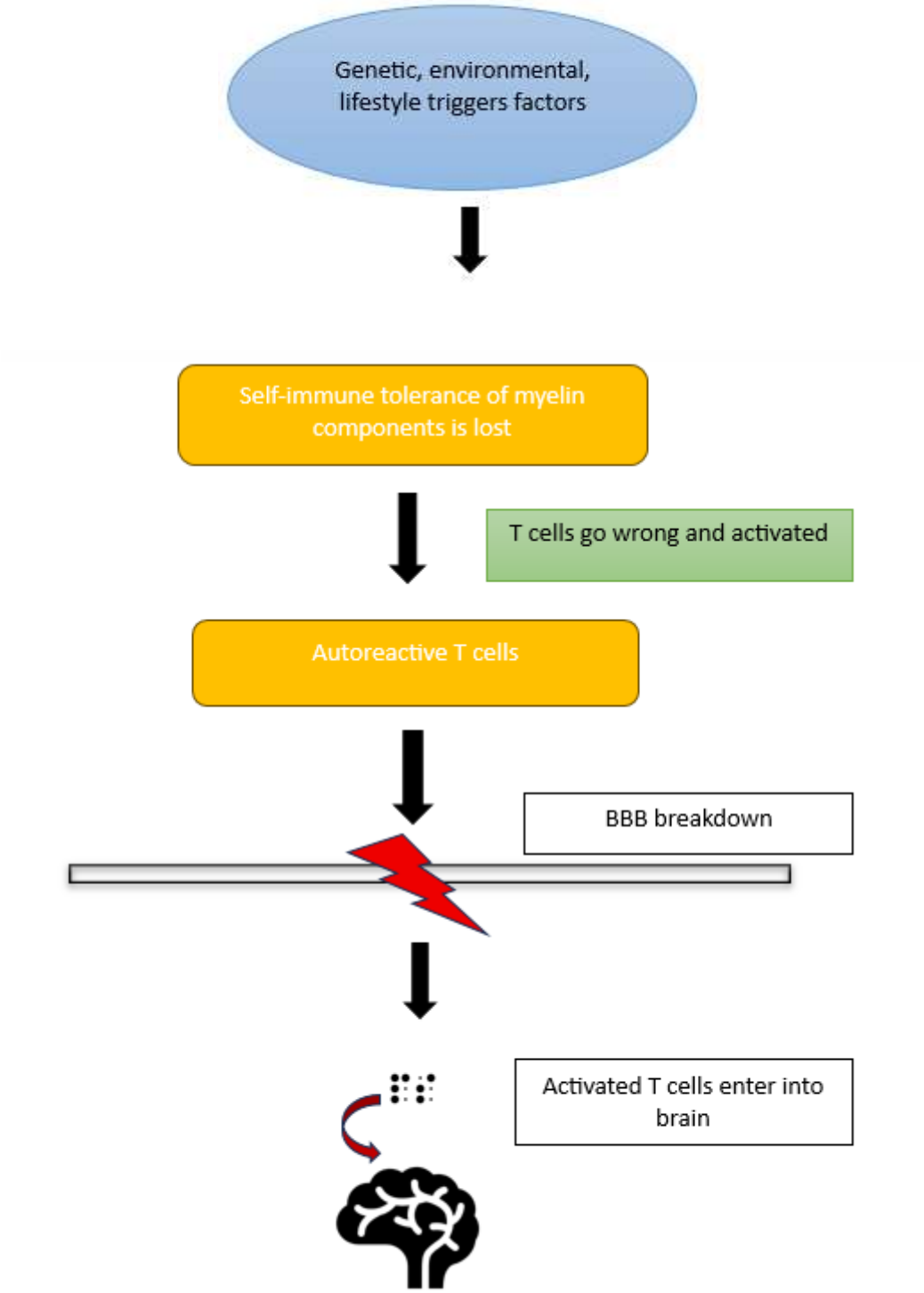
3.1 Immune Dysregulation and Autoimmune Response

Multiple sclerosis occurs due to auto immune reactions that results in demyelination of axons comprising brain and spinal cord (Central Nervous System). The myelin sheath components such as oligodendrocytes, internodes, nodes of ranvier, myelin proteins lost their immunological tolerance and mistakenly affected by own immune cells is the basic pathogenic process causing multiple sclerosis (MS). Under normal physiological circumstances, the immune system can differentiate between self and non-self-antigens and are able to protecting host tissues from immune-mediated harm. However, in case of Multiple sclerosis, this regulating system is compromised, which results in an autoimmune reaction against many important myelin proteins such as Myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP) and are the significant antigens that the immune system targets.

The central nervous system is protected by a highly selective, special physiological barrier that shield the nervous system tissues from immune cells, infections or any poisons present in circulation, which is known as Blood-Brain Barrier (BBB). In multiple sclerosis the structural integrity of BBB is compromised and migration of autoreactive immune cells, especially activated T lymphocytes, across BBB occurs which is a crucial step in the pathophysiology of multiple sclerosis (MS). This event is facilitated by inflammatory mediators and immunological signaling molecules, which permits activated T cells and other immune components to enter the central nervous system. After entering the brain and spinal cord, these autoreactive T lymphocytes recognise myelin antigens and begins an inflammatory cascade. Humongous number of pro-inflammatory mediators like cytokines, chemokines, and other signaling molecules are released as a result of this immunological activation, adding strength to the inflammatory response.

The autoreactive T cells can further recruit other immune cells like B lymphocytes, macrophages, and microglia, while macrophages and activated microglia engage in the phagocytosis and destruction of myelin sheaths, B cells enhance the disease progression by producing antibodies against myelin components. The signal transmission of neurons is hampered by the ensuing inflammatory reactions, which encourages widespread demyelination. Long-term inflammation results in axonal damage and degeneration, which eventually results in irreversible damage and impairment to structure and functions of nervous tissues. Therefore, a pivotal mechanism underlying the pathophysiology of multiple sclerosis

is the breakdown of immunological tolerance to myelin antigens, together with immune cell proliferation, migration and inflammatory signals inside the central nervous system.



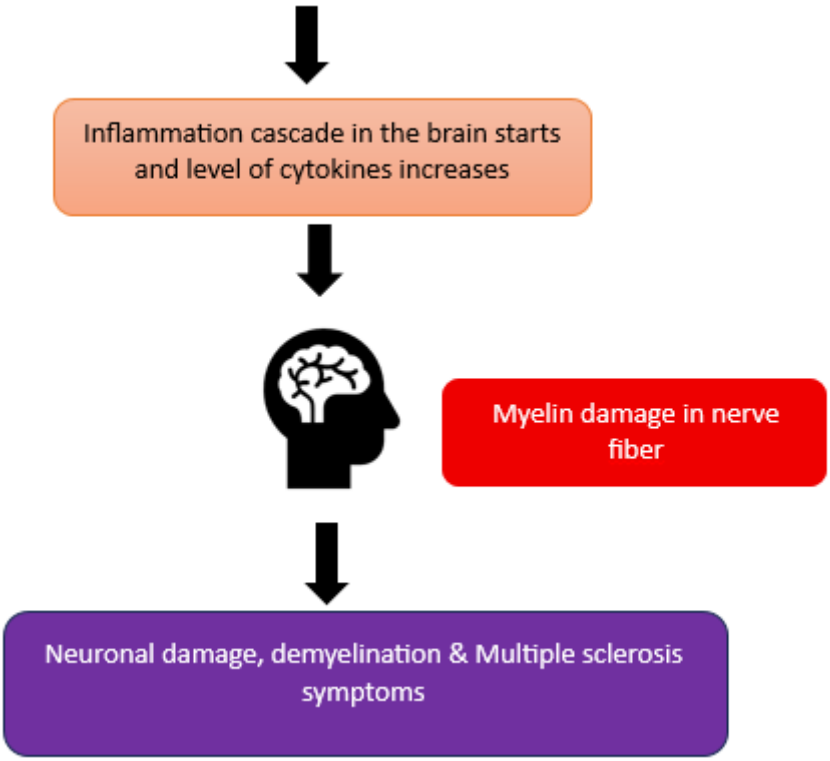


Fig: 1 immune dysregulation in association in MS

3.2 Neurovascular Unit Dysfunction

Nervous system consist of a highly structured and dynamic multicellular system that controls cerebral blood flow and protecting the anatomical and functional integrity of the blood–brain barrier (BBB), and is known as the neurovascular unit (NVU) which is a principal component ensuring proper brain homeostasis. This structure allows the fundamental communication between neuronal, glial, and vascular components of the central nervous system (CNS). This is an integrated network that prevents highly dangerous chemicals and immunological components from entering the systemic circulation along with ensuring the proper oxygen and nutritional supply to cerebral regions. The NVU makes a irreplaceable contribution to the preservation of CNS microenvironment while assuring selective permeability to the BBB.

The interplay and coordinated functioning of structural units present in NVU regulated the particle movement across BBB, cerebral capillary blood flow, metabolic reactions and neurovascular communication. The neurovascular unit is structurally made up of a number of specialized cell types such as structural element of the blood-brain barrier, endothelial cells, pericytes and astrocytes along with which the crucial immune cell, microglia, keep an eye on the neuronal environment and support inflammatory and immunological responses.

To add to it, Oligodendrocyte, a specialized glial cell having numerous extensions also have functional connection with NVU due to their role in neuronal metabolic support and signal conduction, oligodendrocytes and are in charge of creating and maintaining the myelin sheath surrounding neuronal axons.

If there occur any disruption of this coordinated interactions that can impair the integrity of the blood-brain barrier and changing the control of cerebral blood flow. In pathological circumstnaces such as MS, Immune-mediated damage and inflammatory processes can impair NVU function and thereby makes the

BBB more permeable, which permits circulating immune cells—such as inflammatory mediators and autoreactive lymphocytes—to enter the central nervous system. These immune components' attack may begin neuroinflammatory cascades that lead to neuronal damage, demyelination, and eventual neurological disability. The pathophysiology and course of multiple sclerosis are therefore thought to be significantly influenced by neurovascular unit dysfunction, point outing the significance of NVU integrity in preserving CNS immunological homeostasis.

3.3 Role of Microglia and Oxidative Stress

Central nervous system contains a predominant resident immune cell, microglia which are critical for not only regulating immune responses but also safeguarding neuronal homeostasis in the brain and spinal cord. Under normal physiology, microglia continuously monitor the brain tissues, removes cellular debris, promoting neuronal repairing, and aiding in synaptic remodeling. However, in response to injury, infection and inflammatory reactions, microglia get activated and experience structural and functional alterations and enhance the inflammatory signaling and immunological defense. The influence of microglial activation in the pathophysiology of several neurodegenerative and autoimmune diseases, including multiple sclerosis (MS), is obviously evident, due to their paramount role in regulating neuroinflammation. The activated microglia can take part in numerous number of functional characteristics depending on the signals in the surrounding microenvironment. The two main functional phenotypes are the M1 (classically activated) and M2 (alternatively activated) phenotypes. The pro-inflammatory profile of the M1 phenotype are intensified by numerous inflammation mediators, including as cytokines, chemokines, and cytotoxic chemicals. conversely, the M2 phenotype is attributed to neuroprotective and anti-inflammatory properties. M2 microglia enhances neural regeneration and remyelination as well as tissue repair, inflammation resolution, and clearing of cellular debris.

The pathogenesis of MS relay on the imbalance between these microglial phenotypes that contributes to excess production of highly reactive chemicals, such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species (RCS)B that aids in generating oxidative damage and stress in brain tissues. Such oxidative damage contributes to demyelination and axonal degeneration, that ultimately interferes with neural signaling, and mitochondrial damage. Furthermore, activated microglia can recruit pro inflammatory that assure long-term neuroinflammation in the central nervous system there by ailment.

Conclusively, these factors uncover the untouched role of oxidative stress and microglial activation in the pathogenesis of neuroinflammatory, neurodegenerative ailment, multiple sclerosis.

3.4 NLRP3 Inflammasome Activation

A functional molecular sensor, NLRP3 is an essential multiprotein signaling complex of the innate immune system, essential for controlling inflammatory responses by initiating immune defense processes .The nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) is an inflammasome which is an adaptor protein called apoptosis-associated speck-like protein that contain a CARD (ASC), and the effector enzyme caspase-1 make up its structural framework. Activation of this protein can cleave and activate pro-inflammatory cytokines, especially interleukin-1 beta (IL-1β) and interleukin-18 (IL-18) which can function as important inflammatory mediators that help the central nervous system (CNS) amplify immunological responses.

Progression of multiple sclerosis is associated with activation of the NLRP3 inflammasome which can trigger inflammatory signaling pathways and recruit more immune cells to the CNS areas that are impacted. Eventually, these activated immune cascade can harm Oligodendrocytes, the cells that produce

and maintain the myelin coating covering neuronal axons that will lead to demyelination as well as axonal damage and neuronal degeneration. These factors all together can result permanent neurological impairment in MS patients.

Recent research reveal that, one of the main triggers of the NLRP3 inflammasome's activation is functional impairment of mitochondria. Along with involvement in essential regulation of cellular metabolism and energy generation, mitochondria are also involved in immunological signaling pathways. Reactive oxygen species (ROS), mitochondrial DNA, and other metabolic intermediates are among the danger-associated molecular patterns (DAMPs) that defective mitochondria might emit in response to cellular stress or metabolic disruption. Mitochondrial dysfunction causes neuroinflammatory reactions that facilitate demyelination and dementia in addition to upsetting cellular energy equilibrium. Thus, the interaction between NLRP3 inflammasome activation and mitochondrial dysfunction is a paramount process that plays an evident role in the pathogenesis of multiple sclerosis and has become a intended target for novel therapeutic approaches.

3.5 Mitochondrial Dysfunction in MS

One of the paramount cellular organelle, mitochondria do an inevitable role in sustaining cellular energy metabolism and controlling a variety of signaling pathways and are vital to the survival and proper operation of cells. They produce the energy currency of the cell Adebosine Tri Phosphate (ATP) through oxidative phosphorylation within the electron transport chain and contribute to the energy needed for a number of physiological processes, such as synaptic transmission, neuronal signaling, and the maintenance of ion gradients across neuronal membranes. Along with bioenergetics, mitochondria are involved in multivarious physiological functions, including modulation of intracellular signaling pathways, calcium homeostasis, and regulation of apoptosis. Proper mitochondrial functioning is essential for maintaining the structural and functional integrity of the neuronal tissues and glial cells.

Axonal impulse transmission and maintenance require humongous amount of energy supply which explains the significant contribution of mitochondrial dysfunction to the pathophysiology and development of multiple sclerosis (MS). The normal physiological functions of neurons and oligodendrocyte like glial cells are affected by the impairment of mitochondrial function by the lack of energy and they are damaged. In addition to that, the proteins, lipids and nucleic acid components of central nervous system also get negatively affected by the mitochondrial dysfunction since the mitochondrial malfunctioning can produce reactive oxygen species and can harm the components by contributing to oxidative stress.

The development and progression of multiple sclerosis is associated with neuronal and axonal degeneration and demyelination which are intimately linked to mitochondrial dysfunction in addition to metabolic disorders. The destruction of Signal transmission throughout the nervous system is related with disruption of axonal transport mechanisms and degeneration of neuronal processes which are considered as the aftereffects of impairment of mitochondrial physiology. Furthermore, mitochondria can synthesize reactive chemicals and signals that are harmful to neuronal tissue that can stimulate pro-inflammatory signaling pathways and can intensify immunological reactions in the central nervous system. Therefore, the major pathological events that occur in multiple sclerosis such as demyelination, neuronal damage, and neurodegeneration, can eventually accelerate development of disease worsening of cognitive impairment in patients and are intensively related with mitochondrial dysfunction.

3.6 Kynurenine Pathway Dysregulation

In the central nervous system, the necessary amino acid, tryptophan is break down through kynurenine

pathway which involve a sequence of enzyme reactions and produce a number of intermediary metabolites known as kynurenine which is the primary metabolic mechanism and is crucial for controlling immunological reactions and brain activity. On the basis of neurological physiological environment and the balance between various pathway intermediates, these metabolites can produce neuroprotective or neurotoxic effects. Kyneurenic acid is derivative that perform neuroprotective property by regulating excitatory neurotransmission and shielding neurons from excitotoxic damage. However, the other metabolite such as 3-hydroxykynurenine and quinolinic acid act as neurotoxic components and cause excitotoxicity, oxidative stress, and neuronal damage.

Due to neuroinflammation and immune hyper intensity reactions in multiple sclerosis, the strict control over the kynurenine pathway is disrupted due to the stimulation of the sequential enzymes involved such as indoleamine 2,3-dioxygenase (IDO), that can disturb the balance between neuroprotective and neurotoxic pathways, by the stimulation by pro-inflammatory cytokines, especially interferon-γ and other immune mediators. This might result in the build-up of neurotoxic substances.

The damage to both neurons and supporting glial cells occurs due to elevated levels of neurotoxic kynurenine metabolites. These metabolites can cause oxidative stress, damage mitochondria, and increase excitotoxicity, by overstimulating glutamate receptors. Eventually the metabolic disruption through kynurenine pathway dysregulation is one of the essential contributing event in the development and progression of neuronal damage, demyelination and axonal damage which can there by impair proper nerve impulse conduction, saltatory conduction, insulation, protection of neurons. Thus, comprehending the function of this metabolic pathway in the pathogenesis of multiple sclerosis may offer important insights into possible treatment approaches meant to restore metabolic balance and shield brain tissues from inflammatory damage.

4. Dihydroorotate Dehydrogenase (DHODH): Structure and Biological Role

The cellular metabolism in nervous system is mediated by a crucial mitochondrial enzyme, dihydroorotate dehydrogenase (DHODH) through the de novo production of pyrimidine nucleotides. This enzyme can catalyze an essential and rate-limiting step in the pyrimidine production pathway that is the oxidation of dihydroorotate to orotate. This reaction and the enzyme hold paramount importance in the neuronal functioning as pyramidine nucleotides are necessary building blocks for the synthesis of DNA and RNA, there by involving transcription, cellular replication, and other metabolic activities of neurons. The highly proliferating cells require DHODH activity that provide a steady supply of nucleotides for nucleic acid synthesis because of its fundamental role.

The enzyme contain 2 structural domains: flavin mononucleotide (FMN) cofactor that present in catalytic domain that possess role in oxidation of dihydroorotate and membrane binding domain that has role in the interaction between the elements of mitochondrial metabolism, which determine the catalytic activity and cellular location of the enzyme. There is a strict correlation between the DHODH and the mitochondrial electron transport chain (ETC). during the catalytic process, Coenzyme Q (ubiquinone) act as an essential carrier in the electron transport chain, receives electrons produced by the oxidation of dihydroorotate. Through this interaction,DHODH successfully connects mitochondrial respiration, cellular energy metabolism, and pyrimidine nucleotide production and this phenomenon emphasizes DHODH's dual function in promoting mitochondrial bioenergetics and nucleotide synthesis.

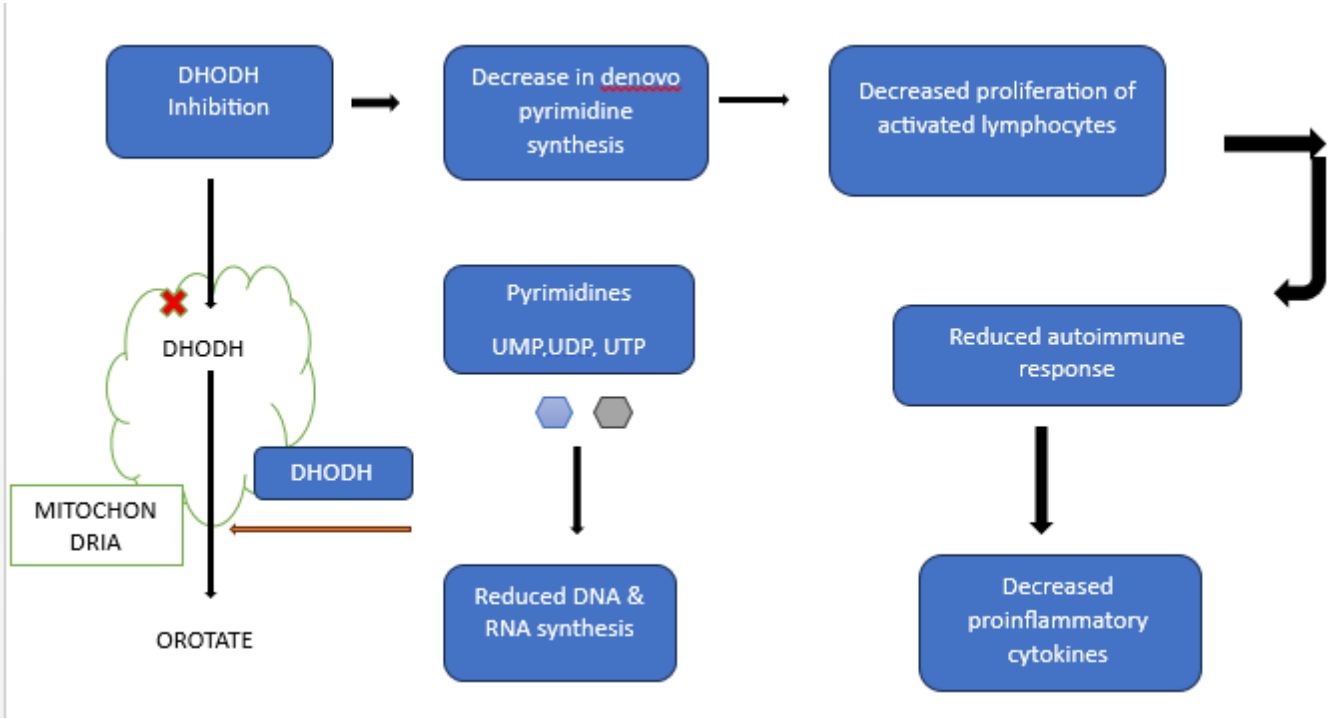
In the multiplication and proliferation of immune cells such as lymphocytes the continuous supply of pyrimidine is required for the DNA replication and transcription of DNA to RNA which clearly describe

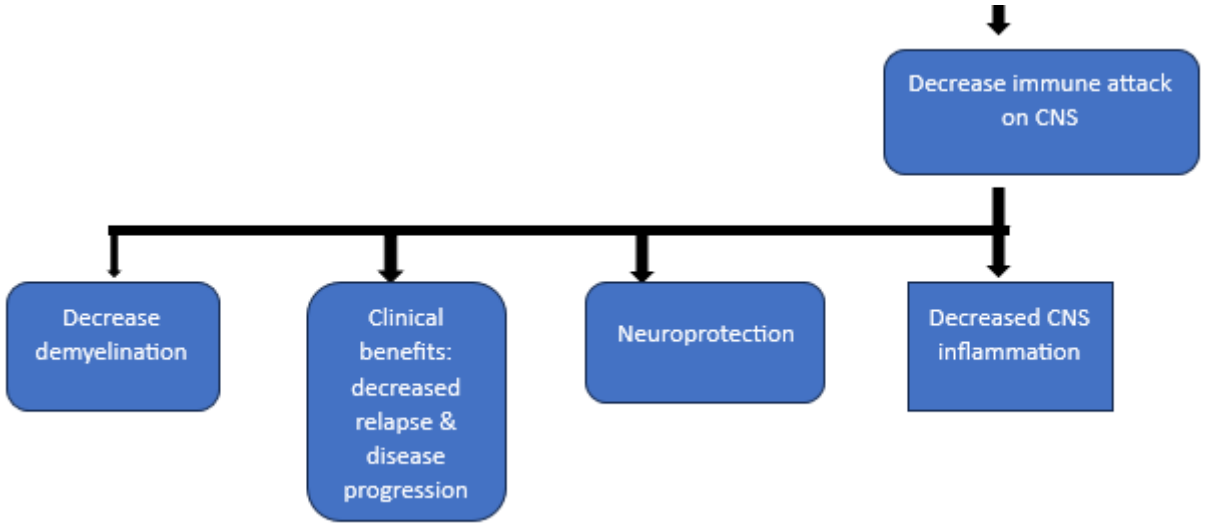
the relevance of DHODH in the immune responses that carried out in autoimmune diseases such as multiple sclerosis. Since the activated lymphocytes primarily depends on the de novo pyrimidine synthesis route to maintain their fast proliferation cheking of DHODH retard the proliferation of these rapidly proliferating immune cells by curbing the availability of pyrimidine nucleotides. This is the reason behind the emergence of novel strategies of targeting DHODH for immunomodulatory therapy especially in multiple sclerosis as the disease pathogenesis involve the active immune cell proliferation, multiplication and recruitment.

Mechanism of Action of DHODH Inhibitors in Multiple Sclerosis

The DHODH inhibitors can disrupt the de novo pyrimidine biosynthesis pathway, which is necessary for the synthesis of nucleotides required for the synthesis of DNA and RNA and there by reduce the availability of pyrimidine nucleotides such as uridine monophosphate (UMP) and stops conversion of dihydroorotate to orotate. The decreased provision of nucleotides can negatively affect Rapidly growing immune cells, such as activated T lymphocytes and B lymphocytes, because the nucleotides mandatory of maintenance of cellular replication and clonal expansion during immune responses.

DHODH inhibition can chiefly inhibit activated T and B cell proliferation which can inhibit triggering autoimmune responses in multiple sclerosis that lead to retardation of the breakdown of myelin sheaths in the central nervous system. The inhibition of DHODH prevents the generation and release of pro-inflammatory mediators such as cytokines, interleukins, interferons, and tumor necrosis factor along with inhibiting lymphocyte proliferation and can reduce immune-mediated tissue damage and attenuates inflammatory signaling pathways.





The DHODH inhibition is related with reduction of autoimmune-mediated demyelination and which is significant effect of DHODH inhibition and can decrease the course of illness by reducing the inflammatory cascades that cause demyelination and brain damage by restricting the activity and growth of these harmful cells.

Normal resting cells are not affected by Inhibition of DHODH but can reduce the iimune responses in activated immune cells due to reduced supply of pyrimidine by denovo synthesis which lowers the danger of broad immunosuppression and preserves the defence capacity of body to fight infections.

6. Currently Approved DHODH Inhibitors

6.1 Teriflunomide is a disease-modifying medication (DMT) that may be used orally and is authorized to treat relapse types of multiple sclerosis. The mitochondrial enzyme Dihydroorotate Dehydrogenase (DHODH), which is essential for the de novo production of pyrimidine nucleotides, is specifically inhibited by this active metabolite of leflunomide.

Teriflunomide prevents the production of uridine monophosphate, a necessary precursor for the creation of DNA and RNA, by inhibiting DHODH activity. This effect mostly impacts immune cells that divide quickly, especially activated T-lymphocytes and B-lymphocytes, whose proliferation is largely dependent on the de novo pyrimidine synthesis pathway.

Teriflunomide is effectual therapeutic agent that can reduces the immune-mediated inflammatory signals that makes demyelination and neuronal damage in multiple sclerosis by curbing the s phase stage of lymphocyte cell cycle. Vitally, this agent can inhibit the pyrimidine salvage pathway, which selectively checks pathogenic immune activation while preserving basic immune function.

Long term investigations and clinical trials possess various therapeutic advantages for multiple sclerosis.Teriflunomide has emerged as a significant treatment option for the long-term management of relapse multiple sclerosis due to its immunomodulatory mechanism and easy oral administration, especially in patients who need long-term suppression of autoimmune-mediated inflammation.

7. Emerging Next-Generation DHODH Inhibitors

7.1 Vidofludimus Calcium (IMU-838)

Vidofludimus calcium is second-generation inhibitor of Dihydroorotate Dehydrogenase (DHODH), and is recently being investigated for a treatment for several autoimmune diseases including multiple sclerosis.

This medication possess better pharmacological and molecular selectivity than teriflunomide by minimizing side effects and enhancing the therapeutic efficacy.

The second generation DHODH inhibitor strongly and selectively binds to the DHODH enzyme that decreases the proliferation of lymphocyte without interfering with other cellular enzymes.

Vidofludimus calcium is predicted to have fewer side effects compared to other DHODH inhibitors, due to its advanced pharmacokinetic actions and increased selectivity. It is being anticipated that vidofludimus calcium can be an efficacious next generation immunomodulatory treatment fo all autoimmune diseases such as multiple sclerosis due to its pharmacological benefits. The safety, efficacy and long term positive effects of the drug in reducing immune mediated inflammation and faltering the disease course are being analysed in ongoing clinical investigations.

8. Therapeutic Applications of DHODH Inhibitors Beyond Multiple Sclerosis

8.1Spinal Cord Injury

Spinal Cord Injury (SCI) is a severe neurological condition characterized by damage to the spinal cord that results in partial or complete loss of motor, sensory, and autonomic functions below the level of injury. In addition to the immediate mechanical damage caused by trauma, a cascade of secondary injury mechanisms occurs after the initial insult. These secondary processes include oxidative stress, inflammation, mitochondrial dysfunction, excitotoxicity, and different forms of regulated cell death, which collectively contribute to progressive neuronal and glial damage.

Recent research has highlighted the involvement of the mitochondrial enzyme Dihydroorotate Dehydrogenase (DHODH) in protecting neural tissues following spinal cord injury. DHODH is primarily known for its role in the de novo pyrimidine synthesis pathway, where it catalyzes the conversion of dihydroorotate to orotate during nucleotide biosynthesis. However, emerging evidence indicates that DHODH also participates in maintaining mitochondrial redox balance and cellular metabolic stability, which are essential for neuronal survival after injury.

One of the key mechanisms through which DHODH exerts its protective effects involves the suppression of Ferroptosis, a recently recognized form of regulated cell death. Ferroptosis is characterized by iron-dependent accumulation of lipid peroxides within cell membranes, leading to oxidative damage and eventual cell death. In the context of spinal cord injury, excessive iron accumulation, mitochondrial dysfunction, and increased lipid peroxidation can trigger ferroptosis in neurons and glial cells, thereby worsening tissue damage and neurological deficits.

Studies suggest that DHODH can limit lipid peroxidation within mitochondria and reduce ferroptotic cell death, thereby helping to preserve neuronal integrity following spinal cord trauma. By modulating mitochondrial metabolism and oxidative stress pathways, DHODH may contribute to neuroprotection and improved functional recovery after injury.

Therefore, understanding the relationship between DHODH activity and ferroptosis regulation may open new therapeutic possibilities for spinal cord injury. Targeting DHODH-related pathways could potentially help reduce neuronal loss, attenuate oxidative damage, and enhance neuroregeneration, making it an emerging area of interest in neuroprotective drug development.

8.2 Cancer Therapy

Dihydroorotate Dehydrogenase (DHODH) inhibitors have garnered significant attention as possible anticancer treatment drugs in recent years. Tumor cells that proliferate quickly depend heavily on the de novo pyrimidine synthesis pathway for growth and survival because they need a lot of nucleotides to

sustain ongoing DNA and RNA synthesis. These inhibitors reduce the supply of nucleotides required for tumor cell growth by interfering with pyrimidine synthesis via inhibiting DHODH. Therefore, DHODH inhibition can slow the spread of cancer in a number of ways. According to studies, it can cause cell cycle arrest, which stops tumor cells from advancing through crucial stages of the cell division cycle.

Furthermore, enhanced activation of the tumor suppressor protein p53, which encourages DNA damage responses and death in aberrant cells, has been linked to DHODH inhibition. Additionally, it could cause ferroptosis, a controlled kind of iron-dependent cell death marked by lipid peroxidation that helps get rid of cancerous cells. DHODH inhibitors have been shown to improve antitumor immune responses in addition to their direct effects on tumor cells. This helps the immune system identify and eliminate cancer cells. When taken as a whole, these pathways demonstrate the possibility of DHODH inhibitors as viable options for the creation of cutting-edge anticancer treatments.

9. Future Perspectives and Emerging Biomarkers

Dihydroorotate Dehydrogenase (DHODH)-targeting treatments have shown promising therapeutic results, but a number of issues still need to be resolved to maximize their clinical efficacy. In order to improve patient selection and therapeutic effectiveness, future research should concentrate on finding trustworthy predictive biomarkers that can assist in determining which patients are most likely to react to DHODH-targeted treatment. Furthermore, more effective and selective DHODH inhibitors must be developed in order to maximize efficacy while reducing toxicity and side effects.

Investigating combination therapy approaches, especially with immunomodulators or metabolic regulators, which may have synergistic effects and enhance disease management, is another crucial line of inquiry. Additionally, thorough long-term safety assessments are required to have a better understanding of the possible negative consequences linked to extended DHODH inhibition. It is anticipated that the therapeutic potential of DHODH inhibitors will be further developed and increased with continued advancements in precision medicine and sophisticated molecular diagnostic technologies, resulting in more efficient and customized treatment methods for a variety of disorders.

10. Conclusion

Multiple sclerosis is a complex autoimmune disorder characterized by immune-mediated demyelination, neuroinflammation, and progressive neurological damage. Although several disease-modifying therapies are available, many treatments remain limited by incomplete efficacy and long-term safety concerns.

Recent advances in immunometabolism have identified **dihydroorotate dehydrogenase (DHODH)** as a promising therapeutic target. By selectively inhibiting de novo pyrimidine synthesis, DHODH inhibitors suppress proliferation of activated lymphocytes while sparing resting immune cells, thereby offering a targeted immunomodulatory approach.

Clinically approved agents such as **teriflunomide** have demonstrated the therapeutic potential of this strategy, while emerging inhibitors such as **vidofludimus calcium (IMU-838)** may provide improved efficacy and safety. Beyond multiple sclerosis, DHODH inhibitors are also being explored for applications in cancer, viral infections, and neurodegenerative disorders, highlighting the broader significance of metabolic regulation in immune-mediated diseases.

Continued research into the molecular mechanisms, clinical efficacy, and biomarker-guided application of DHODH inhibitors will be essential for advancing next-generation therapies and improving long-term outcomes for patients with multiple sclerosis.

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