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DERLEME/REVIEW

Systemic Effects of Methotrexate upon the Peripheral Nerve Tissue

Metotreksatın Periferik Sinir Dokusu Üzerindeki Sistemik Etkileri

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ABSTRACT
Methotrexate (MTX) is a widely used oncologic drug due to its antineoplastic and anti-inflammatory effects. Although its toxic effects on the central nervous system at high doses have long been known, its effects on the peripheral nervous system are less studied in the literature. This review discusses the systemic effects of MTX on peripheral nerve tissue at histopathological, biochemical, molecular, and behavioral levels. Experimental animal studies have shown that MTX administration leads to impaired nerve conduction, reduced myelin thickness, axonal degeneration, and increased glial activity. Moreover, elevated homocysteine levels due to disrupted folate metabolism, oxidative stress, the release of pro-inflammatory cytokines, and mitochondrial dysfunction are thought to be the main mechanisms underlying MTX-induced neurotoxicity. MTX-related peripheral neuropathies are often irreversible in clinical practice, but early diagnosis and appropriate pharmacological interventions may allow for recovery. This review aims to raise awareness by compiling current data on the effects of MTX on the peripheral nervous system, both in basic science and clinical applications.
Keywords: Drug toxicity, methotrexate, myelopathy, peripheral nerve ınjuries, neurotoxicity.

ÖZET
Metotreksat (MTX), hem antineoplastik hem de antiinflamatuvar etkileri nedeniyle yaygın olarak kullanılan bir onkolojik ilaçtır. Yüksek dozlarda kullanıldığında merkezi sinir sistemi üzerinde toksik etkiler gösterebildiği uzun süredir bilinmesine rağmen, periferik sinir sistemi üzerindeki etkileri literatürde daha az araştırılmıştır. Bu derlemede, MTX'in periferik sinir dokusu üzerine olan sistemik etkileri histopatolojik, biyokimyasal, moleküler ve davranışsal düzeylerde ele alınmıştır. Deneysel hayvan çalışmalarında, MTX uygulamasının sinir iletiminde bozulma, miyelin kalınlığında azalma, aksonal dejenerasyon ve glial aktivitede artışa yol açtığı gösterilmiştir. Ayrıca folat metabolizmasının bozulmasıyla artan homosistein seviyeleri, oksidatif stres, proinflamatuvar sitokinlerin salınımı ve mitokondriyal disfonksiyonun, MTX'e bağlı nörotoksisitenin altında yatan başlıca mekanizmalar olduğu düşünülmektedir. MTX ile ilişkili periferal nöropatiler klinikte çoğu zaman geri dönüşsüz olmakla birlikte, erken tanı ve uygun farmakolojik müdahalelerle iyileşme mümkün olabilmektedir. Bu derleme, MTX'in periferik sinir sistemi üzerindeki etkilerine dair güncel verileri bir araya getirerek, hem temel bilim hem de klinik uygulamalar açısından farkındalık yaratmayı amaçlamaktadır.
Anahtar kelimeler: İlaç toksisitesi, metotreksat, miyelopati, periferik sinir yaralanmaları, periferik nöropatiler, nörotoksisite.

Introduction

Methotrexate (MTX) is one of the folate antagonists, an antimetabolite that inhibits the enzyme dihydrofolate reductase, disrupting DNA synthesis, repair, and cellular replication. It is widely used in chemotherapy protocols for various malignancies, as well as in autoimmune diseases such as rheumatoid arthritis (RA) and psoriasis[1,2]. Despite its clinical efficacy, it is known that MTX can cause various neurotoxic side effects in the central and peripheral nervous systems[1]. These neurotoxic effects can present in a wide

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clinical spectrum, including acute or subacute encephalopathy, demyelinating myelopathy, cranial neuropathies, epileptic seizures, cognitive dysfunction, and peripheral neuropathy. MTX-induced neurotoxicity is associated with a multifactorial pathogenesis and is influenced by variables such as the route of administration (e.g., intrathecal vs. systemic), cumulative dose, genetic polymorphisms affecting folate metabolism, and concomitant use of other neurotoxic agents[2].

Evidence on MTX effects in the PNS is fragmented and limited compared with the CNS[3-6]. Most studies rely on rodent models, while clinical evidence largely consists of case reports/series and small observational cohorts[7-11]. Marked heterogeneity exists across dose (chronic low-dose rheumatologic vs. high-dose/intrathecal oncologic), route of administration[4,12], endpoints (nerve conduction/electrophysiology, histopathology, behavioral assays), and follow-up; standardized reporting is uncommon[13-15]. The MTX-specific peripheral neuropathy phenotype—axonal vs. demyelinating, root vs. distal fiber predominance—remains insufficiently defined, and reversibility as well as long-term outcomes are uncertain[3,7]. This gap hampers early recognition, risk stratification, and the development of preventive/therapeutic strategies in clinical practice[5,16].

Our review makes this gap explicit and synthesizes the scattered evidence with a dedicated PNS focus: (i) histopathological, biochemical, and molecular effects of MTX in the PNS; (ii) findings spanning experimental models to clinical phenotypes; (iii) pathomechanisms including folate cycle disruption, homocysteine elevation, oxidative stress, mitochondrial dysfunction, and glial responses; and (iv) diagnostic, monitoring, and treatment implications. In doing so, we map the scope, quality, and blind spots of PNS-focused evidence. This review comprehensively addresses the current literature on MTX-induced neurotoxicity, considering pathophysiological mechanisms, clinical phenotypes, and experimental models.

Methotrexate and its Mechanism of Action

Methotrexate (MTX) is an antimetabolite with antifolate activity, used as both an anticancer and anti-rheumatic drug. In 1947, it was discovered that the folic acid analogue aminopterin induced remission in children with acute lymphoblastic leukemia (ALL), leading to its use. Later, researchers sought other folic acid analogues, and in 1950, MTX, also known as amethopterin, was introduced for the treatment of ALL. By 1951, it was also used for the treatment of RA and psoriasis, and in 1988, it was approved by the Food and Drug Administration (FDA) for RA treatment. While MTX is used at high doses for solid tumors and cancers, it is administered at low doses chronically for chronic autoimmune inflammatory diseases. MTX is also used in osteosarcomas, non-Hodgkin lymphomas, and some types of acute myeloblastic leukemia[17].

MTX competitively inhibits the enzyme dihydrofolate reductase (DHFR), thereby disrupting the production of tetrahydrofolate (THF). The decrease in THF synthesis leads to reduced purine nucleotide and thymidylate synthesis, which in turn decreases cell replication and DNA synthesis. Through this mechanism, MTX exerts its anticancer effects. Its anticancer efficacy is primarily cytotoxic to rapidly proliferating cells, such as lymphocytes. However, the role of MTX in RA treatment is explained differently. MTX reduces purine synthesis, leading to the accumulation of adenosine, which inhibits T cells, decreases the expression of intercellular adhesion molecules by T cells, down-regulates B cells, increases CD95 sensitivity in T cells, reduces methyltransferase activity, and decreases the binding of interleukin-1 beta to the cell surface. These anti-cytokine effects are thought to be responsible for its effectiveness in treating the autoimmune disease RA. It is recommended to be used once or twice weekly for RA treatment[18].

MTX has side effects including hepatotoxicity, intestinal toxicity, nephrotoxicity, cognitive impairment, peripheral neuropathy, axonopathy, and demyelination. These side effects may be due to folate deficiency, which disrupts purine and pyrimidine metabolism[19]. Due to these adverse effects, dose adjustment and sometimes drug discontinuation may be required. This can lead to the interruption of treatment in many patients. Neuropathy and demyelination can persist for a long time in the patient[20]. While numerous studies have been conducted on the effects of MTX on the central nervous system (CNS)[21], there is insufficient research in the literature regarding its effects on peripheral nerves. The neurotoxic effects of MTX are shown in Table-1.

Table 1. Neurotoxic Effects of MTX

Neurotoxicity	Clinical presentations
Acute (Within a few hours)	Drowsiness, mental confusion, exhaustion, impaired orientation, convulsions Chemical-induced arachnoiditis: headache, nausea, vomiting, elevated temperature, back pain, lightheadedness
Subacute (Following days to weeks)	Encephalopathy: one-sided limb weakness, impaired coordination, speech issues, epileptic seizures, confusion, mood swings Myelopathy: leg pain, altered sensation, paraplegia, bladder dysfunction
Chronic (Following months to years)	Learning disability, intellectual impairments, reduced cognitive abilities Leucoencephalopathy: disorientation, drowsiness or agitation, convulsions, lack of coordination, cognitive decline, speech difficulties, full-body paralysis, vision problems, slurred speech, coma, death

MTX:Methotrexate

Methods – Evidence Classification

We pre-specified a two-tier synthesis to avoid conflation of clinical and preclinical data. Evidence from human participants was analyzed under Clinical Evidence, while animal/ex vivo/in vitro studies were analyzed under Preclinical (Animal) Evidence. Mixed reports were split by tier. Within each tier, outcomes were harmonized by domain (clinical: neurological phenotype, NCS/EMG, imaging, longitudinal recovery; preclinical: behavioral assays, electrophysiology/nerve conduction, histopathology, and molecular readouts). Dose and route (low-dose rheumatologic vs. high-dose/intratecal oncologic; intratecal/intravenous/intraperitoneal/subcutaneous) were extracted a priori. Translational comparisons were restricted to a dedicated section.

Clinical Evidence (Human data)

MTX-induced neurotoxicity can occur due to idiosyncratic reactions or a lowered threshold for neuronal damage. MTX-induced leukoencephalopathy is a chronic side effect resulting from neuronal damage. Subacute myelopathy, another MTX side effect, progressively causes paraplegia, sensory loss, and urinary and fecal incontinence[22].

Peripheral neuropathy is commonly observed with the use of chemotherapeutic agents. As the duration and dose of chemotherapy increase, the severity of peripheral neuropathy may also intensify[10]. In the study by Yılmaz et al., increased GFAP immunoreactivity in response to MTX was associated with damage to Schwann glial cells in peripheral nerves[11]. Bax/Bcl2 genes play a role in the regulation of apoptosis[23]. The degradation of damaged mitochondria is mediated through the PINK1 and Parkin pathways. Dysfunction in these mitochondrial degradation pathways leads to toxicity and neuronal loss. Both apoptosis and mitochondrial degradation pathways are essential for the healthy functioning of cells[24]. A study reported that in MTX-treated rats, the increase in the Bax/Bcl2 ratio, indicative of an apoptotic effect, activated apoptotic pathways[11]. A study conducted between 1990 and 2021 on patients receiving MTX therapy found that 5.22% of patients developed neurotoxicity. Of those who developed neurotoxicity, 37% received intrathecal MTX, 22.2% received intravenous MTX, and 40.7% received a combination of both. Among neurotoxicity presentations, encephalopathy was the most common (69.2%), followed by encephalomyelopathy (15.4%), myelopathy (11.5%), and polyradiculopathy (3.8%). Pediatric age group, male gender, and receiving intrathecal treatment in adults and intravenous treatment in children were identified as risk factors for neurotoxicity. Brain imaging results indicated that subcortical and deep white matter were most frequently affected (54.55%), followed by the centrum semiovale (40.91%) and periventricular white matter (36.36%). Spinal involvement was seen in 37.88% of neurotoxic patients, with dorsal column lesions reported in approximately half of these cases[6].

MTX-induced neurotoxic side effects can include epileptic seizures, focal neurological deficits, stroke-like episodes, myelopathy, radiculopathy, posterior reversible encephalopathy syndrome, leukoencephalopathy, and diffuse encephalopathy[25]. MTX can damage the nervous system through astrocytosis, axon loss, and demyelination[26]. It is believed that the neurotoxic effects of MTX are due to its inhibition of dihydrofolate reductase. This inhibition raises homocysteine levels and lowers methionine levels, which in turn increases the levels of excitatory sulfur-containing amino acids and adenosine while decreasing tetrahydrobiopterin (BH4) levels. These changes lead to numerous alterations in neuronal pathways. A meta-analysis has associated peripheral neuropathy with elevated homocysteine levels[27]. In a study of diabetic patients, an increase in homocysteine levels was linked to diabetic neuropathy[28]. The increase in homocysteine and the reduction in S-adenosylmethionine (SAM) levels, which is used in myelin sheath synthesis, are thought to explain MTX's neurotoxic effects[29].

MTX disrupts purine synthesis, leading to the accumulation of adenosine[30]. The breakdown of adenosine occurs via the enzyme adenosine deaminase (ADA). Increased ADA activity has been observed in immunoinflammatory and metabolic diseases. In conditions such as myasthenia gravis, Graves' disease, RA, and systemic lupus erythematosus, serum ADA activity has been elevated. Inflammatory diseases like ectopic pregnancy, preeclampsia, inflammatory bowel disease, and gestational diabetes have also shown elevated ADA levels[31]. A study in diabetic patients identified high ADA levels as a risk factor for neuropathy. Those with high ADA levels were found to have lower nerve conduction speed[32].

Acute neurotoxic symptoms related to MTX can present as stroke[33] or transverse myelitis[34]. In pediatric patients, epileptic seizures may occur[35]. MTX-induced myelopathy can present as subacute combined degeneration (SCD), though it differs in that it does not respond to cobalamine[6]. In a study of 13 leukemia patients treated with intrathecal MTX, urinary and bowel incontinence, motor weakness, sensory loss, and dorsal column hyperintensity on MRI were observed, resembling SCD. However, normal vitamin B12 levels in these patients confirmed the differential diagnosis. In 8 patients with available data, 7 showed elevated serum homocysteine and low serum folate levels[36].

Dextromethorphan can be used for MTX-induced neurotoxicity. Dextromethorphan blocks N-methyl-D-aspartate (NMDA) receptors non-competitively and plays a protective role against the neurotoxic effects of homocysteine and other excitatory amino acids[22]. Peripheral neuropathies associated with MTX have been reported at the case-report level. In a patient with central nervous system lymphoma receiving high-dose intrathecal MTX, weakness and hyporeflexia in the right leg improved after MTX discontinuation[37].

In two patients treated with intrathecal MTX, urinary retention followed by lower limb weakness and subsequent paraplegia was observed. Electromyography (EMG) showed no F waves, and magnetic resonance imaging (MRI) revealed spinal cord involvement. The patients were diagnosed with acute lumbar polyradiculoneuropathy. Despite discontinuing MTX and adding methylprednisolone, there was no improvement in their neurological condition[7]. MRI in MTX-induced myelopathy may show dorsal hyperintensity[38]. In cerebrospinal fluid, an increase in myelin basic protein levels may be observed[39].

MTX-induced transverse myelopathy is a rare condition that can arise from intrathecal MTX therapy. In transverse myelopathy, which causes isolated spinal cord dysfunction, symptoms appear within hours or days after MTX treatment without compressive lesions. Factors that increase the risk of MTX-induced transverse myelopathy include high-dose intrathecal MTX therapy, systemic MTX treatment, active CNS disease, MTX treatment intervals of less than one week, and the use of other chemotherapeutic drugs[40]. Only about 3% of patients receiving intrathecal MTX develop transverse myelopathy[41]. MTX-induced myelopathy can affect the lumbosacral level and proximal motor roots, typically resulting in a poor prognosis. There are case reports suggesting that folic acid supplementation can improve symptoms of MTX-induced myelopathy[42].

Steroid therapy can be used to reduce vasogenic edema in MTX-induced neurotoxicity[43]. In addition to dextromethorphan, leucovorin[44] and aminophylline[45] are also used for this purpose. Various drugs have been experimentally studied to reverse MTX-induced neurotoxicity. Ketamine, with its NMDA antagonistic effect, has been tried in patients with MTX-induced neurotoxicity who require sedation[46].

The literature also mentions the occurrence of lymphoproliferative diseases in the spinal cord associated with MTX. It has been suggested that high-dose MTX can activate Epstein-Barr virus, which causes lymphoproliferative diseases through apoptotic mechanisms and B-cell transformation. Case reports have shown that discontinuation of MTX therapy leads to regression of these lymphoproliferative diseases[12,47].

Intrathecal MTX is widely used to prevent CNS relapse in ALL patients. A case report described a 31-year-old male patient with T-cell ALL who developed progressive paraplegia within days after intrathecal MTX infusion. Pathological examinations revealed transverse necrosis and extensive macrophage infiltration in the thoracic spinal cord, along with subpial vacuolar degeneration in the cerebellum and lumbar regions, and loss of cerebral white matter[48]. Additionally, MTX's effects on folate metabolites, particularly 5-methyl-THF and SAM, impair myelination and cause degeneration in both the CNS and peripheral nervous system[49]. Furthermore, MTX's direct toxic effects on endothelial cells can lead to damage of the vessel walls, allowing the drug to penetrate deeply into the CNS parenchyma, resulting in necrosis and vacuolar degeneration[50]. If not treated, this toxicity can lead to rapid neurological symptoms and permanent motor loss. In this case, a diagnosis of MTX-induced transverse myelopathy was made[48], and this side effect occurs in approximately 3% of cases[51]. High-dose steroids and folic acid analogs are recommended in the presence of this side effect[48].

MTX inhibits the DHFR enzyme, lowering THF levels and disrupting the synthesis of essential compounds for DNA and RNA synthesis, such as purines and thymidine. This reduction in THF production disrupts the homocysteine-methionine cycle, leading to elevated homocysteine levels and decreased methionine levels[52]. In healthy individuals, cerebrospinal fluid (CSF) homocysteine levels are typically ≤0.5 nmol/mL, but after systemic MTX treatment, these levels can rise to 1.0 nmol/mL[53]. Elevated homocysteine levels and decreased methionine activate excitatory mechanisms through NMDA receptors, which contribute to neurotoxicity[54]. Another component of MTX toxicity is the dysfunction of astrocytes, which leads to axonal loss and demyelination, particularly in the dorsal columns of the spinal cord[55]. Clinical signs of SCD in patients receiving high-dose systemic and intrathecal MTX therapy include progressive paraplegia, sensory loss, and neurogenic bladder. Genetic factors, such as polymorphisms in methylenetetrahydrofolate reductase (MTHFR), may modulate sensitivity to MTX neurotoxicity, but their role remains unclear. Despite being considered irreversible, MTX-induced myelopathy has shown improvement with early intervention, including folate supplementation, SAM, dextromethorphan, and intensive rehabilitation therapy[56]. These findings highlight the complexity of MTX neurotoxicity and the need for developing optimal therapeutic strategies to mitigate these effects through specific molecular and genetic mechanisms.

Peripheral neuropathy associated with subcutaneous MTX administration has also been reported. In a 70-year-old female patient with RA, damage to the median nerve was observed after subcutaneous MTX injection, which improved with pregabalin treatment[57]. In a study by Zhou et al. in rats, short-term MTX treatment led to a significant increase in calcitonin gene-related peptide (CGRP)-positive nerve fibers in the tibial periosteum, which was parallel to the development of pain behaviors triggered by touch in the following days. Mechanical allodynia, assessed using von Frey tests, was significantly increased in the MTX group, suggesting that MTX lowered the pain threshold at peripheral nerve endings. These effects may be due to MTX's disruption of folate metabolism, increasing homocysteine levels, triggering neuroinflammation, and causing hyperexcitability in sensory neurons. Additionally, MTX may promote the remodeling of sensory fibers in tissues such as the periosteum, increasing sensitivity to painful stimuli. These findings suggest that MTX can cause neuropathy in the peripheral nervous system through both direct toxic and indirect inflammatory mechanisms, and that chemotherapy-induced pain may be linked not only to bone damage but also to increased sensory innervation[10].

Preclinical (Animal) Evidence

In a 2024 study, rats were divided into four groups: control, MTX, agmatine, and MTX+agmatine. Rats received 37.5 mg/kg/week of intraperitoneal MTX for 3 weeks. The study found that MTX-treated rats exhibited longer escape times in the water maze test, reduced time spent in the quadrant, fewer frames crossed in the open field test, increased nociceptive latencies, and decreased nerve conduction speed and sciatic function index. Histological examination of the sciatic nerve in the MTX group revealed a decrease

in myelin thickness and axon diameter. Additionally, increased glial fibrillary acidic protein (GFAP) immunoreactivity was observed in the sciatic nerve of the MTX-treated rats. Western blot analysis showed increased Bax/Bcl2 protein expression, but no changes in Parkin expression. The MTX+agmatine group showed significant improvement in the peripheral neuropathy caused by MTX. The prolonged escape times in the water maze test suggest that MTX also affected the rats' learning and memory abilities[11].

BH4 is essential for normal brain maturation. A study in rats with congenital heart disease showed an association between reduced BH4 levels and delayed brain maturation[58]. Other studies reported that exogenous BH4 administration corrected hypoxia-induced delays in myelination, improved sensorimotor coordination, and reduced apoptosis in white matter[59]. BH4 is also required for the hydroxylation of phenylalanine, tyrosine, and tryptophan, thus influencing dopamine and serotonin synthesis. Due to the biochemical reactions induced by MTX, the reduction in dopamine and serotonin levels leads to symptoms such as hypokinesia, body hypotonia, difficulty swallowing, oculogyric crisis, limb rigidity, and recurrent hyperpyrexia[60].

In an experimental rat study investigating the histological, immunohistochemical, and biochemical effects of MTX, hippocampal structural damage, degeneration of pyramidal cell layers, cerebellar congestion, and Purkinje cell degeneration were observed. Increased caspase-3 expression in the hippocampus and cerebellum was also noted[61]. Caspase-3 is known for its pro-apoptotic effect and for marking the irreversible point of apoptosis[62]. Furthermore, GFAP immunoreactivity was increased in the hippocampus and cerebellum of MTX-treated rats[42]. GFAP, a cell skeleton filament, appears in the CNS following cell damage or cell death[63].

A study examining the effects of MTX on peripheral nerves using carvacrol and pomegranate in rats found increased levels of total oxidants, MDA, Tumor necrosis factor (TNF)-alpha, and interleukin-1-beta, while total antioxidant levels were decreased in the sciatic nerves of MTX-treated rats[64]. TNF-alpha and interleukin-1-beta are pro-inflammatory cytokines associated with cell death and inflammation[65]. It is believed that MTX has anti-inflammatory effects at low doses, while at higher doses, it triggers the release of pro-inflammatory cytokines, leading to neurotoxicity[66].

Previous studies have shown that MTX administration leads to oxidative stress in various tissues, including the sciatic nerve, spinal cord, and brainstem[61,64,67]. MTX-associated neurotoxicity is primarily linked to the increased production of reactive oxygen species (ROS), leading to lipid peroxidation and cellular damage. The role of oxidative stress in MTX-induced neurotoxicity has been highlighted in studies investigating potential neuroprotective effects of antioxidants like caffeic acid phenethyl ester (CAPE). Known for its antioxidant properties, CAPE significantly reduced levels of malondialdehyde (MDA), a marker of oxidative stress, and improved the activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) in neuronal tissues. These findings suggest that MTX-induced damage could be alleviated with CAPE, making it a promising agent to reduce MTX-related peripheral nerve toxicity[67].

In a study by Pranaya et al. (2021), the effects of *Passiflora incarnata* and pregabalin were evaluated in MTX-induced neuropathy. MTX was administered to rats to induce peripheral neuropathy, leading to behavioral changes such as increased thermal hyperalgesia (sensitivity to heat) and cold allodynia (pain from non-painful stimuli). Pain sensitivity was elevated in the MTX group. Biochemical markers of oxidative stress, including TBARS (a marker of lipid peroxidation), GSH (reduced glutathione), and calcium levels, were measured in the sciatic nerve tissue. MTX treatment increased TBARS and calcium levels while decreasing GSH levels. *Passiflora incarnata* treatment reversed these changes, demonstrating the plant's antioxidant and neuroprotective properties. Histopathological analysis of the sciatic nerve after MTX treatment revealed axonal degeneration and swelling, but treatment with *Passiflora incarnata* significantly reduced these pathological changes, showing a protective effect on the nerve tissue[68].

In a study by Scholz et al. (2008), it was shown that at one-fiftieth of the dose that induces neurotoxicity, the spinal microglial activation and subsequent neuropathic pain behaviors following peripheral nerve injury could be suppressed. In this study, rats with a spinal nerve injury model were given low-dose intrathecal MTX, which significantly reduced microglial activity in the dorsal horn, suppressed p38 mitogen-activated protein kinase (MAPK) phosphorylation, and reversed pain behaviors such as mechanical and cold

allodynia. The study emphasized that this effect was observed only when treatment was initiated early and had no significant impact on microglial activation when applied later. These findings suggest that MTX might play a role not only in its neurotoxic effects but also in neuropathic processes due to its immune-modulatory potential[69].

Translational Bridge

We confine cross-tier interpretation to this section. Convergence: Across tiers, folate-cycle disruption (↑homocysteine; ↓methylation capacity), oxidative stress, glial responses, and axonal/myelin injury recur. Divergence: Clinical phenotypes cluster around intrathecal/high-dose exposures with variable reversibility, whereas preclinical models emphasize reproducible molecular and glial signatures under controlled dosing. Bridging metrics: standardized NCS/EMG endpoints, explicit reporting of dose/route and time-to-onset, and homocysteine/S-adenosylmethionine panels may improve translation. A potential bidirectional window exists wherein low-dose regimens modulate neuroimmune pathways (e.g., microglia), while higher doses drive toxicity reinforcing dose timing context in clinical decision-making.

Conclusion

MTX, with its potent therapeutic effects, also draws attention due to its toxic effects on the nervous system. As presented in this review, MTX-induced neurotoxicity is not only related to the suppression of folate metabolism but also involves multifactorial mechanisms such as oxidative stress, pro-inflammatory responses, mitochondrial dysfunction, glial cell activation, and apoptosis. These effects in the peripheral nervous system can lead to significant changes both at the behavioral level as well as histopathologically and molecularly. The findings suggesting that MTX may suppress microglial activation at low doses to prevent the development of neuropathic pain imply that the drug's immunomodulatory effects could create a delicate balance between toxicity and therapy, depending on the dose. Therefore, when planning MTX therapy, the patient's neurological risk profile should be considered, and careful monitoring is essential, especially with high doses and intrathecal administration. Future studies will contribute to a better understanding of these mechanisms, aiding in the development of targeted therapies to prevent or reverse MTX-induced neurotoxicity.

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