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SNI: Trauma

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Review Article

Neuroprotection or Neurotoxicity? Insights from preclinical and clinical studies of methotrexate in spinal cord injury

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Received: 28 August 2025
Accepted: 25 December 2025
Published: 20 February 2026

DOI
10.25259/SNI_953_2025

Quick Response Code:

Supplementary Materials available from:
http://dx.doi.org/ 10.25259/SNI_953_2025

ABSTRACT

Background: Spinal cord injury (SCI) remains a serious condition with limited treatment options. Methotrexate (MTX), an immunosuppressant with anti-inflammatory and neuroprotective properties, has shown promising effects in experimental SCI, but clinical observations in oncology patients raise concerns regarding neurotoxicity. The aim of this study was to systematically evaluate the effect of MTX on SCI in preclinical models and to compare these findings with available human evidence.

Methods: A comprehensive literature search was conducted in PubMed, Scopus, and Web of Science up to the end of 2024. Animal studies investigating MTX in SCI were included. Data extraction and quality assessment were performed independently by two reviewers. A random-effects meta-analysis was conducted where appropriate. Human evidence, including large-scale retrospective data, was synthesized for interpretation.

Results: Six animal studies (five rats, one rabbit) met the inclusion criteria. MTX administration consistently improved functional recovery, reduced apoptosis, decreased oxidative stress, and improved preservation of myelinated axons. Meta-analysis demonstrated a significant reduction in myeloperoxidase activity (standardized mean differences = –3.40, 95% confidence interval [CI] –5.73 to –1.08, I^2 = 91.18%). In contrast, clinical evidence, mostly from oncology patients with intact spinal cords, consistently reported MTX-associated neurotoxicity, including encephalopathy, dorsal column myelopathy, and polyradiculopathy. The largest cohort identified a 5.2% incidence of neurotoxicity, with a higher risk in children and intrathecal (IT) administration.

Conclusion: Preclinical evidence supports MTX as a potential neuroprotective agent in SCI, but human data demonstrate neurotoxicity in noninjured spinal cords, especially with IT use. Differences in route of administration, dosing, host condition, and injury context likely explain this contrast. IT MTX should not be considered in traumatic SCI. Future studies should define safe systemic dosing windows in injured spinal cord models and evaluate cautious early-phase clinical trials under strict monitoring.

Keywords: Methotrexate, Neuroprotection, Spinal cord injury, Systematic review

INTRODUCTION

Spinal cord injury (SCI) remains one of the most serious neurological conditions, characterized by severe motor, sensory, and autonomic dysfunction.[18,24] With a global incidence ranging from 10.4 to 83 cases per million people annually, it predominantly affects young individuals, leading to significant morbidity and mortality.[1,5,13] The complex pathophysiology of SCI involves both

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primary mechanical trauma and secondary injury cascades, including inflammation, oxidative stress, and apoptosis, which collectively contribute to permanent neurological deficits.[17,25,33] Despite advances in medical care, current treatment options remain limited, creating an urgent need for effective therapeutic interventions.[3,8]

Methotrexate (MTX), a well-established immunosuppressant and anti-inflammatory agent widely used in rheumatoid arthritis and other autoimmune conditions,[24] has emerged as a potential therapeutic option for SCI.[18] Its ability to modulate immune responses and inflammation, combined with its well-documented safety profile and Food and Drug Administration (FDA) approval status, makes it an attractive candidate for using in SCI treatment.[14,26] Recent experimental studies have suggested that MTX may have neuroprotective properties and could potentially alleviate secondary injury mechanisms in SCI.[8]

The therapeutic potential of MTX in neurological conditions has been demonstrated through various experimental models.[3,8,14,18,24,26] Several studies have investigated its effects on reducing neutrophil infiltration,[26] oxidative stress,[14] and apoptosis[24] in injured spinal cord tissue. Furthermore, research has suggested that MTX may have protective effects on myelinated axons[14,26] and could promote functional recovery following SCI.[8,18] However, the collective evidence regarding its efficacy and mechanisms of action in SCI has not been systematically evaluated.

This systematic review and meta-analysis aims to comprehensively evaluate the available evidence regarding the therapeutic potential of MTX in SCI for the 1st time. By analyzing data from multiple experimental studies, we seek to assess the effects of MTX on functional recovery, tissue preservation, and various pathophysiological mechanisms involved in secondary injury. This analysis will provide valuable insights into the potential clinical application of MTX in SCI treatment and identify areas requiring further investigation.

MATERIALS AND METHODS

Study design

This systematic review and meta-analysis were performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 guidelines[22] to evaluate the effect of MTX on SCI individuals. The study was designed with the help of the PIT format: population: individuals or animal models with SCI. Intervention: administration of MTX. Target Condition: injured spinal cord tissue.

Search strategy

A comprehensive literature search was conducted across multiple electronic databases, including Medline (through PubMed), Scopus, Web of Science, and Embase, covering publications up to the end of 2024. The search strategy was developed using keywords and synonyms related to MTX and SCI. To ensure thoroughness, a manual search was also performed using the Google search engine and by examining the references of relevant articles. The complete search strategy employed in this study is available in Supplementary Material 1.

Inclusion and exclusion criteria

We included peer-reviewed articles that examined the effect of MTX in SCI individuals. The exclusion criteria were as follows: studies without outcome assessments, lack of a control group of untreated SCI individuals, studies of combination therapies that did not include MTX alone, reviews, abstracts, and duplicate publications. Human evidence, including large-scale retrospective data, was synthesized for interpretation.

Data collection

Records from the databases were exported to EndNote (version 21; Thomson Reuters, Toronto, ON, Canada), and duplicates were removed. Two independent reviewers then screened the titles and abstracts, and any disagreements were resolved by a third reviewer. Following this initial screening, the full texts of potentially eligible articles were thoroughly reviewed based on the inclusion and exclusion criteria. Data from the selected articles were extracted into a predefined Excel spreadsheet. This spreadsheet included various parameters such as study characteristics (author, publication year, and design), population details (species, strain, sex, age, and weight), SCI induction characteristics (model of injury and details of interventions including timing, dosage, frequency, and administration route), and outcome measures which encompassed motor functional recovery, biochemical markers (inflammation, oxidative stress, and apoptosis), and histological analysis (neuronal damage and apoptosis).

Quality assessment

The risk of bias in the included studies was evaluated using the Systematic Review Centre for Laboratory Animal Experimentation Risk of Bias tool.[12] All the signaling questions of each domain were answered separately, and finally, a conclusion was drawn. These evaluations were also done by two independent reviewers, and disagreements were resolved through discussions with the third reviewer.

Statistical analyses

Statistical analyses were performed using Stata 17.0

(StataCorp LLC, College Station, TX, USA). We synthesized the results from various studies and reported pooled effect sizes as standardized mean differences (SMD) with a 95% confidence interval (CI). Given the methodological and clinical heterogeneity among the included studies, a random-effects model was applied. We assessed heterogeneity between studies using I^2 and Chi-square tests. Meta-analysis was conducted only when data were available from at least three separate analyses across three distinct studies.

RESULTS

Characteristics of the included studies

The systematic search yielded 1463 nonduplicate records, of which 14 underwent full-text review. The study selection process is illustrated in Figure 1. Six animal interventional studies met the inclusion criteria, while no eligible human studies were found. Five studies used rat models (three SD and two Wistar), and one used rabbits. The SCI models included contusion (four studies), compression (one), and ischemic reperfusion (one). MTX dosing varied widely, with initial doses ranging from 0.05 to 30 mg/kg and total doses from 0.05 to 150 mg/kg. Treatment frequency ranged from single dose to seven doses. Three studies used intraperitoneal (IP) administration, two used intravenous (IV), and one used intrathecal (IT). Treatment initiation ranged from immediately post-SCI to 4 h post-SCI, with five studies starting immediately. Follow-up periods spanned from 1 day to 8 weeks. A detailed description of the characteristics of these studies is provided in the table 1.

Preclinical studies

Functional recovery

The effect of MTX on functional recovery in SCI models was assessed in four studies using various tests and parameters. Gezici *et al*. evaluated motor function scores in rats, demonstrating significant improvement in MTX-treated animals compared to untreated SCI rats from day 1 postinjury.[8] Kertmen *et al*. used the Tarlov score in rabbits, showing significantly higher scores in the MTX group compared to the ischemia group 1 day after injury.[14] Liu *et al*. assessed multiple gait parameters in rats, revealing significant improvements in the MTX therapy group at 8-week post-SCI. These improvements included decreased swing time, stride time, and minimum longitudinal deviation, as well as increased instant run speed, footprint area, and regularity index.[18] Rong *et al*. evaluated functional recovery in rats using the Basso, Beattie, and Bresnahan (BBB) scale and inclined plane test, reporting significantly higher scores in the MTX group compared to the untreated SCI control group at 7- and 14, 5 days following SCI.[24] Collectively, these studies consistently demonstrate that MTX therapy enhances functional recovery in various SCI models across different species and assessment methods.

Neural damage

The neuroprotective effect of MTX was assessed ultrastructurally in two studies. In Kertmen *et al*.'s rabbit ischemia-reperfusion model, injury increased damage scores for all axon sizes at day 1. MTX therapy significantly improved scores for small and medium myelinated axons but not for large ones.[14] In Sanli *et al*.'s rat contusion model, injury worsened scores for large and medium axons, while small axons were unaffected. MTX therapy (both 0.5 mg/kg and 1 mg/kg doses) significantly improved scores for large and medium myelinated axons.[26] These studies suggest that MTX has a protective effect on myelinated axons, with some variations based on the injury model.

Apoptosis

Apoptosis was assessed in three studies examining the effects of MTX on SCI, with one study evaluating apoptotic cell numbers and two studies measuring caspase-3 activity, a pro-apoptotic enzyme. In a rat contusion model, Gezici *et al*. found that MTX treatment significantly reduced the number of apoptotic cells compared to the untreated SCI group at day 10 postinjury.[8] Kertmen *et al*. demonstrated that MTX administration decreased caspase-3 activity compared to the untreated ischemia group in a rabbit ischemia-reperfusion SCI model at day 1 postinjury.[14] Similarly, Rong *et al*. showed that MTX treatment resulted in decreased caspase-3 activity compared to the untreated SCI group in a rat contusion model at day 7 postinjury.[24] These findings consistently indicate that MTX treatment reduces apoptosis in various SCI models.

Neutrophil infiltration and inflammation

Myeloperoxidase (MPO) is a marker of neutrophil infiltration and inflammation. A meta-analysis of 3 studies comparing MPO levels in treated vs. untreated SCI groups showed that MTX administration significantly decreased MPO levels (SMD = −3.40 [95% CI: −5.73 to −1.08], I^2 = 91.18%) [Figure 2]. This suggests that MTX reduces neutrophil infiltration and inflammation in SCI.

Oxidative stress

Malondialdehyde (MDA) and lipid peroxidation (LPO) levels are tissue oxidative stress markers. Three separate studies evaluated the effects of MTX on these markers in SCI models. Bakar *et al*. used LPO levels in a rat contusion model, finding that MTX decreased oxidative stress in the subacute

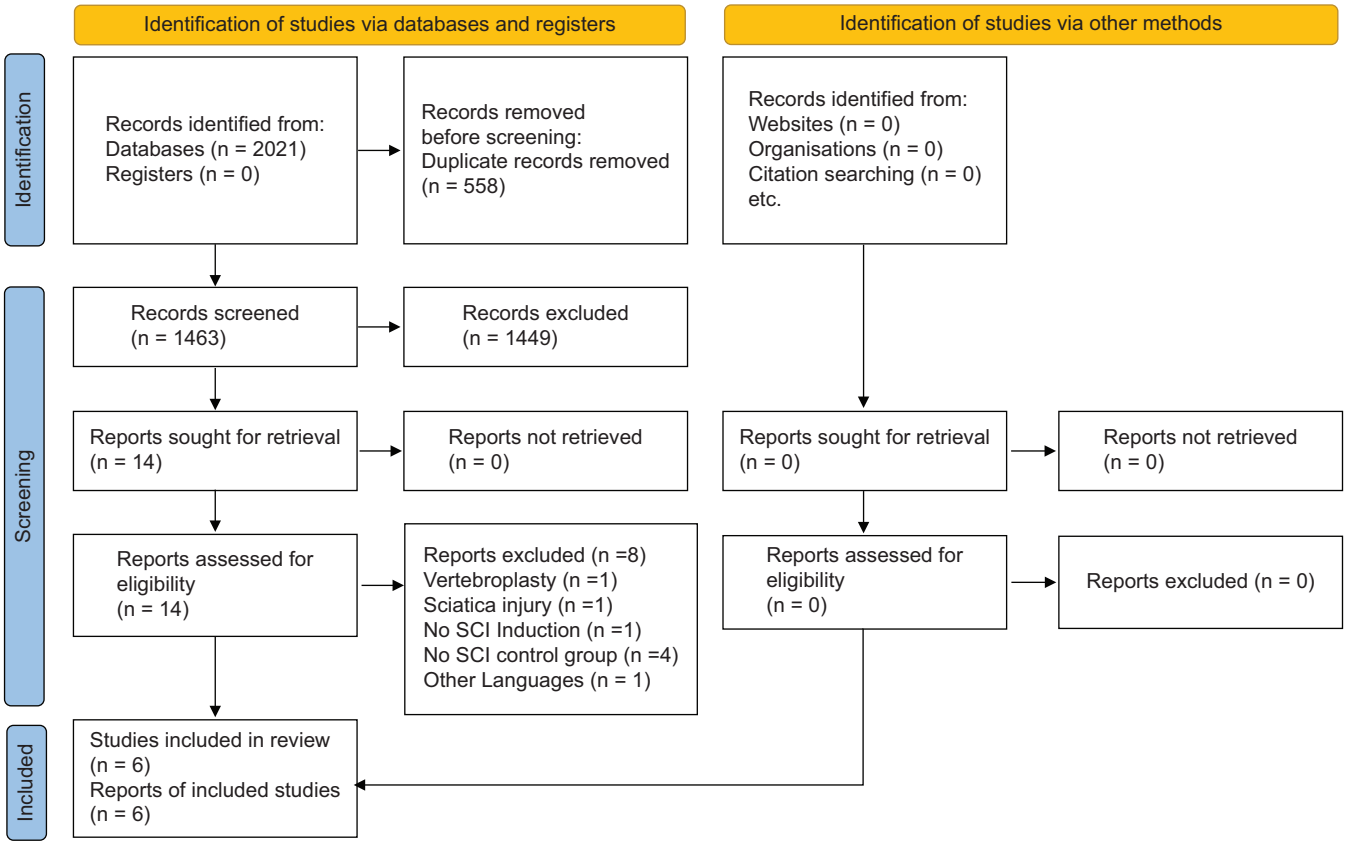

Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram of the screening process.

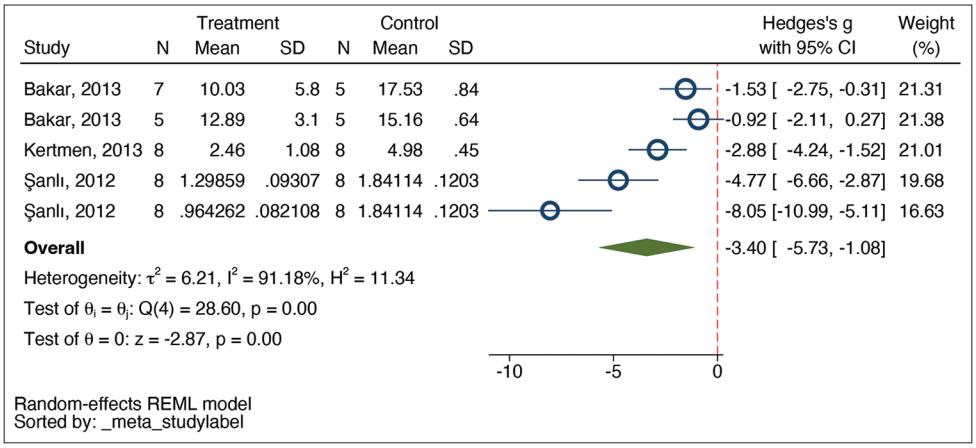

Figure 2: The forest plot for the efficacy of methotrexate in reducing myeloperoxidase as a marker of neutrophil infiltration and inflammation. SD: Standard deviation, CI: Confidence interval.

stage (5-day post-SCI).[3] Kertmen *et al.* and Sanli *et al.* both used MDA levels in their studies. Kertmen *et al.* showed that MTX administration reduced tissue MDA levels compared to the untreated ischemia group on day 1 postinjury.[14] Similarly, Sanli *et al.*'s rat contusion model demonstrated that both low-dose and high-dose MTX groups had significantly lower MDA levels compared to the untreated SCI group on day 1 postinjury.[26] These studies collectively suggest that MTX effectively reduces oxidative stress in SCI models.

Risk of bias assessment

The sequence generation was clearly reported as low risk in only 1 out of 6 studies,[26] while it remained unclear in the other 5 studies. All studies demonstrated low risk of bias regarding baseline characteristics. However, none of the studies provided clear information on allocation concealment, leaving this aspect unclear across all studies. Random housing was consistently implemented, showing

low risk of bias in all studies. Blinding of trial caregivers was not explicitly addressed in any of the studies, remaining unclear throughout. Similarly, random outcome assessment was not mentioned in any study, leaving this aspect unclear. Blinding of outcome assessors was reported in 4 studies.[3,8,14,26] while it was unclear in 2 studies.[18,24] All studies showed low risk of bias for incomplete outcome data, selective outcome reporting, and other sources of bias [Table 2].

Clinical studies

To date, no randomized controlled trials or prospective clinical studies have directly evaluated the use of MTX in the management of traumatic SCI in humans. However, important indirect evidence is available from oncology patients exposed to IT MTX, which provides insights into potential neurotoxic risks when MTX reaches the spinal cord roots or parenchyma.

A large retrospective cohort study including 498 cancer patients treated with MTX systematically evaluated the incidence and characteristics of MTX-related neurotoxicity. Overall, 26 patients (5.22%) developed clinically significant neurological complications. Neurotoxicity was more frequent in children (7.44%, 18/242) compared with adults (3.13%, 8/256), with children above 10 years showing the highest risk (14.5%). Male sex was also associated with

Table 1: Summary characteristics of the included studies.

Author, Year	Gender, strain, species, weight (g)	Number of controls/ treated	SCI model	Initial dose (mg/kg),totaldose (mg/kg), route of administration	Treatment timing, treatment frequency	Follow-up (Days)
Bakar *et al.*,[3] 2013	NM, Rat, Wistar, 250–350	5/-5-7	Contusion	0.05, 0.05, IP	4 h post-SCI, Single dose	3–5
Gezici *et al.*,[8] 2017	Female, Rat, SD, 250–300	8/8	Compression	0.05, 0.05, IP	Immediate, Single dose	10
Kertmen,[14] 2013	Male, Rabbit, New Zealand white, 2800–3550	8/8	Ischemia–reperfusion injury	0.5, 0.5, IV	Immediate, Single dose	1
Liu *et al.*,[18] 2017	Male, Rat, SD, 225–265	10/10	Contusion	0.3, 2.1, IV	30 min post-SCI, 7 doses	56
Rong *et al.*,[24] 2018	Male, Rat, SD, 220–250	8-8	Contusion	50, 150, IT	Immediate, 3 doses	7–14
Şanlı *et al.*,[26] 2012	Female, Rat, Wistar, 250–300	8/8	Contusion	0.5 and 1, 0.5 and 1, IP	Immediate, Single dose	1

IP: Intraperitoneal, IT: Intrathecal, IV: Intravenous, NM: Not mentioned, SCI: Spinal cord injury, SD: Sprague-Dawley

Table 2: Risk of bias assessment of included studies based on the SYRCLE tool.

Study	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding trial caregivers	Random outcome assessment	Blinding outcome assessors	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Bakar *et al.*,[3] 2013	unclear	low	unclear	low	unclear	unclear	low	low	low	low
Gezici *et al.*,[8] 2017	unclear	low	unclear	low	unclear	unclear	low	low	low	low
Kertmen,[14] 2013	unclear	low	unclear	low	unclear	unclear	low	low	low	low
Liu *et al.*,[18] 2017	unclear	low	unclear	low	unclear	unclear	unclear	low	low	low
Rong *et al.*,[24] 2018	unclear	low	unclear	low	unclear	unclear	unclear	low	low	low
Şanlı *et al.*,[26] 2012	low	low	unclear	low	unclear	unclear	low	low	low	low

increased susceptibility. Outcomes were varied widely with 53.8% of patients showing improvement or stabilization, 34.6% showing deterioration, and 11.5% dying during the same hospitalization. Prognosis was generally better for encephalopathy compared with myelopathy or encephalomyelopathy. The median follow-up period was 225 days (range 35–1837 days). This study represents the most comprehensive human evidence to date and highlights a clinically significant risk of MTX-associated neurotoxicity, contrasting sharply with the predominantly protective effects reported in preclinical SCI models.[2]

In addition, several retrospective and case-series reports document MTX-associated myelopathy and polyradiculopathy. Pinnix *et al*. described a cohort of 13 leukemia patients treated with IT MTX who developed dorsal column myelopathy with posterior column T2 hyperintensity on MRI, independent of leukemic infiltration.[23] Cachia *et al*. similarly reported 7 adult patients with IT MTX-induced myelopathy, in whom 5 demonstrated dorsal column lesions.[6] These findings are also supported by case reports. Tariq *et al*. described necrotizing transverse myelopathy,[29] Murata *et al*. reported subacute myelopathy,[21] Gosavi *et al*. documented a posterior column syndrome mimicking subacute combined degeneration,[9] Miyoshi *et al*. reported subacute neuronal damage,[20] while Landolfi *et al*.[16] and Xu *et al*.[31] described anterior lumbosacral polyradiculoneuropathy and cauda equina involvement, respectively.

In summary, these studies indicate that IT MTX can induce clinically significant spinal cord and root pathology, including myelopathy, transverse necrosis, and polyradiculopathy. These adverse outcomes are in significant contrast to the protective effects observed in preclinical models and highlight a serious safety gap that must be addressed before considering clinical use of MTX in SCI.

DISCUSSION

This systematic review and meta-analysis provide compelling evidence for the potential therapeutic effects of MTX in SCI models. The findings suggest that MTX administration may improve functional recovery, reduce neural damage, decrease apoptosis, attenuate inflammation, and mitigate oxidative stress in various experimental SCI models. However, the integration of animal and human data reveals a significant controversy. On the one hand, preclinical models consistently suggest that MTX may serve as a neuroprotective agent following SCI and these effects support the experimental rationale for MTX as a therapeutic candidate. On the other hand, human evidence derived from oncology patients has demonstrated that IT MTX highlights severe neurotoxicity, with multiple reports of myelopathy, transverse necrosis, and radiculopathy. This divergence may be attributable to differences in dosing regimens, routes of administration (systemic in animal models vs. IT in clinical oncology), and disease context. Nonetheless, the clinical data raise critical safety concerns that cannot be ignored.

SCI is a devastating neurological disorder resulting in complete or partial loss of neural functions due to primary mechanical trauma and subsequent secondary injury cascades. SCI progression is divided into two main phases: primary and secondary. The primary phase involves immediate mechanical trauma to the spinal cord, including hyperbending, over-stretching, rotation, and laceration. The secondary phase, more complex and prolonged, is subdivided into acute, subacute, and chronic subphases. The acute subphase involves inflammation, edema, ischemia, hemorrhage, and release of toxic substances. The subacute subphase is characterized by cellular responses such as macrophage infiltration, microglial activation, and astrogliosis, with ongoing inflammation. The chronic subphase involves neuronal apoptosis, axonal retraction and degeneration, demyelination, glial scar formation, and the establishment of permanent sensorimotor deficits. Effective recovery from SCI depends on a multifaceted approach that targets the complex secondary processes while promoting neural repair and regeneration. This comprehensive strategy involves neuroprotection to minimize further damage, neuroregeneration to stimulate axonal regrowth and remyelination, and neuromodulation to optimize remaining neural function.[17,25,33]

The treatment options for SCI remain limited, presenting a significant challenge in clinical practice.[30] This shows the need for novel therapeutic approaches that can effectively address the complex pathophysiology of SCI. In this context, MTX emerges as a promising candidate for SCI treatment. MTX, characterized by its potent anti-inflammatory and immunosuppressive properties, is already a first-line prescription agent for rheumatoid arthritis and has shown encouraging results in various neurological disorders. Its well-established safety profile and existing FDA approval for other indications position MTX as a potential option for SCI treatment.[15,24]

SCI typically results in a marked increase in MPO activity, which is a reliable marker of neutrophil infiltration into the injured tissue. MPO, a specific enzyme abundantly present in the azurophilic granules of neutrophils, plays a crucial role in their oxygen-dependent bactericidal activity. The level of MPO activity is directly associated with both the absolute number of neutrophils and their activation state at the injury site.[26,28] Our meta-analysis provides compelling evidence that MTX treatment significantly reduces MPO levels in SCI models, indicating a substantial decrease in neutrophil infiltration and inflammation. This finding aligns with previous observations in rheumatoid arthritis and psoriasis patients, where MTX rapidly reduced the *in vitro* chemotactic

responses of neutrophils.[7,11] The ability of MTX to mitigate neutrophil infiltration in SCI models suggests a potential mechanism by which the drug may limit the inflammatory cascade and subsequent secondary injury.

Neutrophils, which infiltrate the injured spinal cord tissue, are major sources of free radicals in the extracellular space. These free radicals can trigger LPO, a destructive process that targets the polyunsaturated fatty acids in cell membranes. The chain reaction of phospholipid peroxidation initiated by LPO severely compromises the structural and functional integrity of cell membranes, ultimately leading to cell death. MDA, the most abundant aldehyde resulting from LPO, serves as a reliable marker of oxidative stress.[4,26] By mitigating oxidative stress and inflammation, MTX may contribute to cellular preservation and improved functional outcomes in SCI models. In addition, a previous study observed that MTX led to a more pronounced reduction in LPO levels in the subacute stage of SCI compared to methylprednisolone.[3] This suggests that MTX may be more effective in mitigating oxidative damage during this critical phase of injury.

Analyses have consistently demonstrated MTX's protective effect on myelinated axons, suggesting a preservation of neural tissue integrity. This finding is complemented by a significant reduction in apoptosis observed across studies, indicating MTX's ability to decrease programmed cell death in the injured spinal cord. Apoptosis, a form of programmed cell death, plays a crucial role in SCI's secondary injury cascade. MTX's ability to attenuate apoptotic processes, likely through modulation of mitochondria-associated cell death pathways involving key proteins such as Bcl-2, Bax, and caspases, suggests a mechanism for preserving neurological function. The consistent reduction in apoptosis markers, particularly cleaved-caspase 3, indicates that MTX may interfere with these pathways, promoting cell survival.[10,32] This dual action of preserving myelinated axons and reducing apoptosis demonstrates MTX's potential as a neuroprotective agent in SCI.

The most clinically relevant finding is the consistent improvement in functional recovery observed across multiple studies using different assessment methods. From motor function scores to gait parameters and BBB scales, MTX-treated animals consistently outperformed untreated SCI controls.

While the findings of this systematic review highlight MTX's potential to alleviate oxidative stress and inflammation in SCI models based on preclinical studies, it is essential to acknowledge that there are still controversies in its clinical beneficence in other diseases. Despite its demonstrated neuroprotective properties in SCI in animal models, some studies have reported paradoxical neurotoxic effects in other neurological contexts. For instance, MTX has been shown to induce LPO, leading to oxidative stress in the brain. Notably, studies in rodent models suggest that MTX reduces antioxidant activity and impairs neurogenesis, particularly in the hippocampus and prefrontal cortex regions critical for cognitive function. These findings raise concerns about whether prolonged or high-dose MTX administration could have unintended neurotoxic consequences, particularly in long-term treatment scenarios.[27]

The apparent contrast between preclinical neuroprotection and human neurotoxicity with MTX can be rationalized by considering differences in exposure, context, and methodology across evidence streams:

a) Route and tissue exposure: Most animal SCI studies administered systemic MTX (IP or subcutaneous) at low doses, whereas human data largely utilize high-concentration IT delivery in oncology. IT administration exposes dorsal columns, roots, and ependymal surfaces to peak MTX levels that are unlikely to be achieved with systemic dosing in intact or even acutely injured cords. This pharmacokinetic gradient also explains why animal studies report antioxidative and anti-apoptotic benefits, while human IT reports document dorsal column myelopathy and lumbosacral polyradiculopathy.[2,6,9,16,20,21,23,29,31]
b) Dose–response and rescue: Preclinical signals often arise within a low-dose window consistent with immunomodulation like adenosine-mediated anti-inflammatory effects. In contrast, oncologic IT regimens aim for cytotoxic exposure that more strongly inhibits folate pathways. Absent or delayed folinic acid (leucovorin) rescue, folate depletion, and impaired myelin synthesis may precipitate vacuolar myelopathy and necrosis. Thus, a biphasic response with beneficence at low systemic exposure and toxicity at high IT exposure is a rational mechanistic model.[3,14,18,19,26]
c) Timing and disease context: Animal experiments typically dose within hours of a controlled SCI and track short to intermediate windows (days–weeks), whereas human IT cases accumulate exposure over weeks to months in the setting of leukemia/lymphoma, concomitant neurotoxic agents such as cytarabine, cranial/neuraxial irradiation, and nutritional risks involving folate and B12. These co-factors can amplify susceptibility to MTX neurotoxicity and do not show in the otherwise healthy laboratory animal.[2,8,19,24]
d) Barrier biology and injury physiology: The blood–spinal cord barrier is transiently compromised after SCI, but permeability is heterogeneous across segments and time. Systemic MTX may preferentially reach perilesional inflammatory microenvironments at low levels which is sufficient for immunomodulation without recreating the IT peak in dorsal columns which is involved in human myelopathy. On the other hand, direct IT exposure bypasses endothelial regulation,

producing focal peaks.[6,23]

e) Outcomes and internal validity: Preclinical readouts (MPO, MDA, caspase-3, histology scores, motor scales) are sensitive to short-term biochemical change and may not represent delayed demyelination. Therefore, long-term neurophysiological or imaging correlates including analogous to dorsal column T2 hyperintensity are rarely reported. Risk of bias (limited randomization, allocation concealment, blinding, and small sample size) and publication bias toward positive animal findings may further increase the gap between preclinical and clinical studies.[3,14,18,19,26]

f) Mechanistic reconciliation: At low systemic exposures, MTX may increase extracellular adenosine and reduce microglial activation, endoplasmic reticulum stress, and consequently apoptotic cascades, consistent with the protective signatures reported. At higher or direct exposures without adequate folate rescue, inhibition of thymidylate synthase and disruption of one-carbon metabolism impair oligodendrocyte function and axonal maintenance, producing the posterior column predominant myelopathy observed clinically.[2,9,21,29,31]

Limitations and future directions

Despite these promising findings in preclinical studies, several limitations of the current evidence should be noted. First, the included studies were conducted mostly in rat animal models, with no human studies meeting the inclusion criteria. While animal models are crucial for initial investigations, the translation of these findings to human SCI patients remains to be established. Second, there was considerable heterogeneity in MTX dosing regimens, administration routes, and timing of treatment initiation across studies. This variability complicates the establishment of a standardized treatment protocol, reinforcing the need for further studies to optimize dosing and administration strategies. Finally, the risk of bias assessment revealed some methodological concerns, particularly regarding allocation concealment and blinding of caregivers. Future studies should address these methodological issues to strengthen the reliability of the findings.

Future investigations on MTX in SCI should begin with preregistered, randomized, blinded, and adequately powered animal studies comparing systemic routes (IV, IP, and SC) with IT delivery, coupled with intensive pharmacokinetic sampling of plasma, CSF, and cord tissue to map concentration time profiles against biomarkers such as MPO, MDA, and caspase-3, as well as long-term outcomes such as motor recovery, dorsal column myelin integrity on MRI, and electrophysiology (somatosensory evoked potentials). Studies should include both sexes, varied ages, and comorbid models including SCI with folate/B12 restriction or cytarabine exposure to reflect clinical vulnerability. Defining a therapeutic window by linking cord and CSF exposure to efficacy versus early toxicity, and testing leucovorin rescue schedules to preserve immunomodulatory effects while minimizing folate pathway injury, will be essential. Pharmacology efforts should use physiologically based PK models that integrate barrier changes after SCI and validate target engagement through MTX levels, adenosine metabolites, and neuronal injury biomarkers (glial fibrillary acidic protein and neurofilament light) in large animals. Afterward, the potential for combination therapies, such as using MTX in conjunction with methylprednisolone or other neuroprotective agents, can be explored which may result in synergistic benefits. Early clinical exploration should focus on non-IT, safety-first designs: a randomized, placebo-controlled, dose-escalation trial of low-dose IV MTX in acute SCI (AIS A–C, within 12 h) with strict exclusion criteria, predefined leucovorin rescue, MRI and biomarker safety monitoring, and stopping rules for any signs of myelopathy.

Finally, preregistration of protocols, adherence to animals in research: reporting of *in vivo* experiments and consolidated standards of reporting trials, and open data sharing including neutral results are required to reduce bias. IT MTX should not be pursued; only carefully designed systemic low-dose strategies with robust PK and safety oversight can ethically test its plausibility.

CONCLUSION

Current evidences demonstrate a significant diversity between preclinical and clinical data on MTX in the spinal cord. Experimental studies across multiple animal models consistently suggest neuroprotective effects, including attenuation of oxidative stress, reduced apoptosis, and improved motor recovery following SCI. In contrast, clinical evidence, largely derived from oncology patients receiving IT MTX, demonstrates a pattern of neurotoxicity, with myelopathy, polyradiculopathy, and encephalopathy reported across case series and large cohorts, especially in children and with IT administration.

Overall, these findings show a major gap in knowledge in this matter. The conflict may be explained by differences in route, dosing, pharmacokinetics, host vulnerability, and co-treatments. While low systemic doses may show immunomodulatory and neuroprotective benefits, high or direct exposure particularly IT carries substantial risk of cord injury. Therefore, IT MTX should not be considered in traumatic SCI. Future progress requires detailed preclinical work with clinically relevant models as well as thorough pharmacological studies to define safe therapeutic windows and, if pursued, carefully monitored early-phase trials of systemic low-dose MTX. Only through this cautious and methodical approach can the true therapeutic potential or

limitations of MTX in SCI be clarified.

Ethical approval: The research/study was approved by the Institutional Review Board at Shahid Beheshti University of Medical Sciences (SBMU), number IR.SBMU.RETECH.REC.1401.380, dated September 11, 2022.
Declaration of patient consent: Patient's consent is not required as there are no patients in this study.
Financial support and sponsorship: Nil.
Conflicts of interest: There are no conflicts of interest.
Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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How to cite this article: Azimi A, Vazirizadeh-Mahabadi M, Yarahmadi M, Sarveazad A, Yousefifard M. Neuroprotection or Neurotoxicity? Insights from preclinical and clinical studies of methotrexate in spinal cord injury. Surg Neurol Int. 2026:17:100. doi: 10.25259/SNI_953_2025

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