https://doi.org/10.31925/farmacia.2025.1.14 **ORIGINAL ARTICLE**

PROTECTIVE EFFECT OF TETRAMETHYLPYRAZINE COMBINED WITH METHYLPREDNISOLONE ON THE SPINAL CORD AFTER SPINAL CORD INJURY IN RATS

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Abstract

Spinal cord injury (SCI) is a severe condition associated with significant disability, necessitating research into neuroprotective strategies. This study examined the protective mechanisms of tetramethylpyrazine (TMP) combined with methylprednisolone (MP) in SCI. Thirty-six male SD rats were assigned to six groups: sham, SCI, TMP (200 mg/kg), low-MP (20 mg/kg), high-MP (30 mg/kg), and TMP+MP (200 mg/kg TMP + 20 mg/kg MP), with drug interventions administered for seven days. An acute SCI model was induced using the spinal cord impact method. Motor and sensory functions were assessed using the Basso, Beattie & Bresnahan (BBB) scale and Reuter score, respectively. Serum levels of IL-1β, IL-10, TNF-α, superoxide dismutase (SOD) and malondialdehyde (MDA) were measured, along with histopathological and apoptotic markers (TUNEL staining, caspase-3 expression). Compared to the SCI group, all treatment groups showed improved BBB scores, reduced Reuter scores, decreased neuronal apoptosis, lower IL-1β, TNF-α and MDA, increased IL-10 and SOD, and reduced caspase-3 expression. The high-MP and TMP+MP groups demonstrated the most significant improvements. These findings suggest that TMP combined with MP enhances motor and sensory recovery post-SCI by mitigating inflammation and oxidative stress.

Rezumat

Leziunile măduvei spinării (SCI) reprezintă o afecțiune severă asociată cu un grad ridicat de dizabilitate, justificând necesitatea unor strategii neuroprotectoare eficiente. Acest studiu a investigat mecanismele de protecție ale tetrametilpirazinei (TMP) în combinație cu metilprednisolon (MP) în SCI. Treizeci și șase de șobolani SD masculi au fost repartizați în șase grupuri: martor (sham), SCI, TMP (200 mg/kg), MP doza mică (20 mg/kg), MP doza mare (30 mg/kg) și TMP+MP (200 mg/kg TMP + 20 mg/kg MP), cu administrare timp de șapte zile. Modelul de SCI a fost indus prin impact spinal. Funcția motorie a fost evaluată prin scala Basso, Beattie & Bresnahan (BBB), iar funcția senzorială prin scorul Reuter. S-au determinat nivelurile serice ale IL-1β, IL-10, TNF-α, superoxid dismutazei (SOD) și malondialdehidei (MDA). Histopatologic, s-au evaluat modificările tisulare prin colorația hematoxilină-eozină și apoptoza neuronală prin testul TUNEL și expresia caspazei-3. Comparativ cu grupul SCI, toate grupurile tratate au prezentat îmbunătățiri ale scorului BBB, reducerea apoptozei și markerilor inflamatori, creșterea IL-10 și SOD. Grupurile cu MP doza mare și TMP+MP au avut cele mai bune rezultate. Aceste date sugerează că TMP combinat cu MP îmbunătățește recuperarea motorie și senzorială prin reducerea inflamației și stresului oxidativ după SCI.

Keywords: spinal cord injury, tetramethylpyrazine, methylprednisolone, inflammation, oxidative stress, neuroprotection

Introduction

Spinal cord injury (SCI) is a disorder of the central nervous system characterised by a high disability rate, severely impacting patients' quality of life [1]. Pathophysiological responses, including astrocyte activation, free radical generation, inflammatory responses and axonal conduction failure, occur rapidly after SCI [2]. Methylprednisolone (MP), a synthetic corticosteroid, possesses potent immunosuppressive properties and is widely used clinically in treating SCI [3]. Administering high doses of MP within eight hours of SCI significantly promotes spinal cord (SC) function recovery [4]. Nevertheless, prolonged high-dose MP administration may lead to adverse effects such as gastric mucosal damage, hyperglycaemia, osteoporosis, and femoral head necrosis. Current research has found that traditional Chinese medicine (TCM) also holds certain advantages in treating nerve injuries, with main strategies involving promoting blood circulation and removing blood stasis [5, 6]. Chuanxiong, the dried rhizome of the *Umbelliferae* plant *Ligusticum chuanxiong* Hort., is

a representative blood-circulating and stasis-dispersing herb in TCM, known for its effects of promoting blood circulation, relieving pain, and dispelling wind. Tetramethylpyrazine (TMP) is a biologically active extract found in Chuanxiong [7]. TMP exhibits various pharmacological effects. It can inhibit myocardial contraction, decrease heart rate, and increase cardiac blood flow, exerting a protective effect against myocardial ischaemia-reperfusion injury [8]. TMP can concentration-dependently lower intracellular calcium levels in coronary artery cells and inhibit vascular smooth muscle cell proliferation, thereby increasing coronary blood flow [9]. TMP also possesses anti-tumour properties. Studies have shown that it markedly inhibits the proliferation of colon cancer, prostate cancer, breast cancer cells, and induces apoptosis [10-12]. In addition, TMP also exhibits considerable neuroprotective effects. Guo *et al*. found that TMP could increase the survival rate of midbrain neurones induced by MPP+ and alleviate oxidative stress while maintaining mitochondrial function, further improving motor deficits and neurodegenerative changes in Parkinson's mice [13]. Danduga *et al*. confirmed that TMP could dose-dependently reduce seizure scores, primarily by improving brain oxidative stress, excitotoxicity, and neuroinflammation induced by pentetrazole, exerting neuroprotective effects [14]. These findings indicate that TMP has pharmacological effects such as improving blood circulation, anti-tumour activity, and neuroprotection. Nevertheless, the neuroprotective effects of TMP after SCI still require further confirmation.

In this work, a rat model of acute spinal cord injury was established to analyse the neuroprotective mechanisms of TMP, MP, and their combination after spinal cord injury. The aim is to provide reference data for further exploring the efficacy and mechanisms of TMP in treating acute SCI.

Materials and Methods

Chromatographic conditions for the analysis of TMP

TMP (Shanghai YuanYe Biotechnology Co., Ltd., China) was analysed using a Diamonsil C_{18} column (200 mm × 4.6 mm, 5 μm) with benzoic acid as an internal standard. The chromatographic analysis was conducted with a mobile phase consisting of methanol - 1.5% acetic acid (35:65) at a flow rate of 1 mL/min, column temperature of 30°C, detection wavelength of 294 nm, and an injection volume of 10 μL.

Animals and grouping

Forty male specific pathogen-free (SPF) Sprague-Dawley (SD) rats weighing 180-220 g were selected from Shanghai SLAC Laboratory Animal Co., Ltd., China. They were housed in an animal room at 20 - 25°C with relative humidity of 40 - 70% and were adaptively fed with standard maintenance feed (in accordance with the standard GB14924.3-2010 *Nutrient Composition of Experimental Animal Feeds*). Thirty-six rats were randomly selected and rolled into six groups: Sham group, SCI group, TMP group, Low-dose MP group, High-dose MP group and TMP + MP group, with six rats in each group. The animal experiment was approved by the Ethical Committee of Wenzhou Medical University, Taizhou, Zhejiang, China, and all current guidelines for the use of animals in experiments were followed.

Rats in the Sham group underwent T9 laminectomy surgery without subsequent acute SCI induction. Rats in the SCI group, TMP group, Low-MP group, High-MP group, and TMP+MP group underwent T9 laminectomy surgery followed by acute SCI induction to establish the SCI model. Postoperatively, rats in the sham and SCI groups received daily intraperitoneal injections of an equal volume of saline for seven consecutive days. Rats in the TMP group received daily intraperitoneal injections of 200 mg/kg TMP for 7 consecutive days postoperatively. Rats in the low-MP and high-MP groups received intraperitoneal injections of 20 mg/kg and 30 mg/kg MP (Shanghai Macklin Biochemical Technology Co., Ltd., China), respectively, on the first day postoperatively, followed by daily intraperitoneal injections of an equal volume of saline from days 2 to 7. Rats in the TMP+MP group received intraperitoneal injections of 20 mg/kg MP and 200 mg/kg TMP on day 1 postoperatively, followed by daily intraperitoneal injections of 200 mg/kg TMP from days 2 to 7.

Sports function evaluation

On day 7 after modelling, the experimental rats were placed on a flat, frictional plastic board approximately 10 cm high and 80 cm in diameter. The Basso, Beattie and Bresnahan locomotor rating scale (BBB) [15] was used to assess recovery of limb motor function (MF) in rats, and the Reuter scoring system [16] was used to assess recovery of limb sensory function. Observations lasted five minutes and were scored independently by three individuals, with final scores averaged.

Detection of serum inflammation and oxidative stress indicators

On days 1, 3 and 7 post-modelling, blood samples were collected from the rats' tail veins. After standing at 25°C, samples were centrifuged, and the supernatant was divided into two aliquots. One of them was utilised for enzyme-linked immunosorbent assay (ELISA) to measure the levels of interleukin (IL)-1β, IL-10, and tumour necrosis factor (TNF)-α. According to the instructions provided with the IL-1β, IL-10, and TNF-α ELISA kits (Hangzhou Multi-Sciences Biotech Co., Ltd., China), the following procedure was followed: 300 μL of wash buffer was applied to the enzyme-labelled plate, soaked for 30 seconds, and then discarded. Subsequently, 100 μL of standard solution and 20 μL + 80 μL of serum

sample diluent were applied sequentially to the standard wells and sample wells, respectively. After 50 μL of antibody dilution was applied, the plate was sealed and shaken at 200 rpm for 1.5 hours, then the liquid in the wells was discarded, and the plate was washed. Each well was then supplemented with 100 μL of streptavidin-horseradish peroxidase and shaken at 200 rpm for 0.5 hours. After the liquid was discarded and the plate was washed, 100 μL of chromogenic substrate was applied and incubated in the dark for 30 minutes. Eventually, 100 μL of stop solution was utilised. The absorbance was measured at 450 nm and 570 nm employing a SpectraMax Plus 384 full-wavelength microplate reader (Molecular Devices, USA).

Another aliquot of serum sample was processed according to the instructions of the superoxide dismutase (SOD) and malondialdehyde (MDA) assay kits (Nanjing Jiancheng Bioengineering Institute, China). Working reagents were applied sequentially. For SOD measurement, the mixture was left to stand at 25°C for 10 minutes, and absorbance was measured at 550 nm. For MDA measurement, mixture was heated in a 95°C water bath for 40 minutes, cooled under running water, centrifuged at 4,000 rpm for 10 minutes, and absorbance was measured at 532 nm.

Haematoxylin-eosin (HE) staining observation

On the 7th day post-modelling, the T9-T11 SC segments of rats were harvested, divided into two aliquots, and stored at -80°C for future use. One aliquot of tissue was fixed in 4% paraformaldehyde solution for 5 days. Subsequently, tissue dehydration was performed sequentially using 80%, 90%, 95%, and two rounds of 100% ethanol, followed by two rounds of xylene for tissue transparency, each lasting 30 minutes. Tissues were then embedded and sectioned into 5 μm slices using an RM2235 rotary microtome (Leica Biosystems, Germany). After the slides were incubated at 65°C for 6 h, paraffin sections were obtained. The paraffin-embedded tissues were deparaffinised and rehydrated sequentially utilizing xylene I, xylene II, and 100%, 100%, 95%, and 80% ethanol, followed by water immersion. These sections were then immersed in haematoxylin staining solution (Sigma-Aldrich, USA) for 5 min, rinsed with running water, and differentiated with 1% hydrochloric acid-ethanol solution for 3 seconds. After bluing in running water, sections were stained with eosin (Sigma-Aldrich, USA) for 3 minutes and rinsed with running water. Subsequently, tissue dehydration and transparency were achieved using a gradient of alcohol and xylene solutions. Sections were then mounted with neutral resin and observed for histopathological changes under a BX43 microscope (Olympus, Japan).

TUNEL staining observation

Paraffin sections were routinely deparaffinised and then rinsed with phosphate-buffered saline (PBS) three times for five minutes each. According to the instructions of the TUNEL assay kit (Roche, Switzerland), tissue sections were treated with proteinase K working solution (Beijing Tiangen Biotech Co., Ltd., China) at 37°C for 20 minutes and washed three times. Then 5 μL of TdT enzyme and 45 μL of fluorescein-labelled dUTP mixture were applied and incubated at 37°C for 1 hour, followed by three washes. Next, 50 μL of Converter-POD working solution was applied and incubated at 37°C for 30 minutes, followed by three washes. After application of DAB substrate, sections were counter-stained with haematoxylin and washed with water. Tissue dehydration and transparency were achieved using a gradient of alcohol and xylene solutions. The sections were then mounted in neutral resin and visualised. Three random fields were selected to visualise TUNEL-positive stained cells, and the mean was calculated.

Protein expression detection. Another aliquot of frozen SC tissue was taken and lysed to extract proteins. Protein concentration was determined using the Bicinchoninic Acid Assay protein quantification kit (Shanghai Beyotime Biotechnology Co., Ltd., China). Subsequently, 30 μg of protein samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the Mini-Protein Tetra System (Bio-Rad, USA). After gel electrophoresis, proteins were transferred onto a 0.45 μm polyvinylidene fluoride (PVDF) membrane (Millipore, USA), which was then incubated in a 5% skim milk blocking solution at 25°C for 1 hour with shaking. Following blocking, the membrane was incubated overnight at 4°C with a 1:1000 dilution of primary antibodies against Caspase-3 (Abcam, UK) and β-actin (Shanghai Beyotime Biotechnology Co., Ltd., China). After membrane washing, a 1:2000 dilution of secondary antibodies, goat anti-mouse IgG and goat anti-rabbit IgG (Hangzhou MultiSciences Biotech Biotechnology Co., Ltd., China), was applied and incubated at 25°C for 2 h. Following washing, chemiluminescence detection was performed using an enhanced chemi-luminescence Plus detection kit (Shanghai Beyotime Biotechnology Co., Ltd., China), and the membranes were exposed employing a ChemiDoc XRS+ System gel imaging system (Bio-Rad, USA).

Statistical analysis. All data were presented as mean ± standard deviation and analysed using SPSS 23.0 (IBM, USA) software for statistical analysis. One-way analysis of variance was adopted for comparisons among groups, and post hoc LSD-t tests were performed for pairwise comparisons. A p-value < 0.05 meant statistically significant.

Results and Discussion

Chromatographic examination of TMP

TMP is a colourless needle-shaped crystal with a melting point of 80 ~ 82°C and a boiling point of

190°C. Its molecular formula is $C_8H_{12}N_2$ (Figure 1A). HPLC analysis of TMP, as depicted in Figure 1B, revealed a peak elution around 5 min, with a recovery rate ranging from 99.5% to 100.6%.

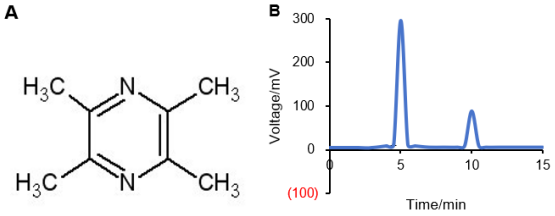

Figure 1.
Determination of TMP
A: chemical structure of TMP; B: HPLC chromatogram of TMP

Evaluation of MF in SCI rats

The MF of rats in each group was assessed using BBB scores, while sensory function was assessed using Reuter scores (Figure 2). Rats in the SCI group showed significantly decreased BBB scores and increased Reuter scores compared to the Sham group ($p < 0.05$). Compared to the SCI group, rats in the TMP, Low MP, High MP and TMP + MP groups showed dramatically increased BBB scores and decreased Reuter scores ($p < 0.05$). In addition, rats in the TMP + MP group showed significantly increased BBB scores, and decreased Reuter scores compared to the TMP and Low-MP groups ($p < 0.05$).

Evaluation of pathological changes in SC of rats with SCI

Histological examination with HE staining was performed to visualise pathological changes in the SC tissues of the rats (Figure 3). In the sham group, only mild oedema was visualised in the SC tissues. In the SCI group, a drastic reduction in the number of anterior horn neurons was observed, along with extensive haemorrhage and infiltration of inflammatory cells in the tissue, as well as the presence of variable sized vacuoles with proliferation of glial cells along the vacuole walls in the grey matter region. In the TMP, Low-MP, High-MP and TMP +MP groups, a reduction in the number of anterior horn neurons and the presence of multiple vacuoles in the grey matter region were observed, although these indicators were less severe compared to the SCI group. The indicators were milder in the High MP group compared to the TMP and Low MP groups, and in the TMP +MP group compared to the High MP group.

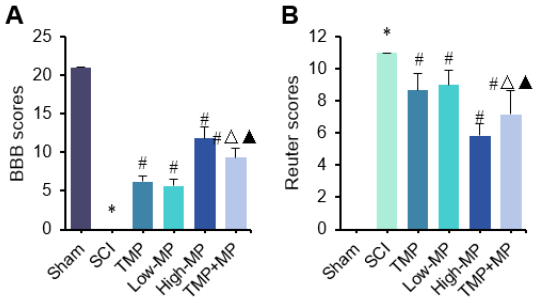

Figure 2.
Evaluation of MF and sensory function in SCI rats across different groups
A: MF scores; B: sensory function scores
*$p < 0.05$ *vs.* Sham group; #$p < 0.05$ *vs.* SCI group; Δ$p < 0.05$ *vs.* TMP group; ▲$p < 0.05$ *vs.* Low-MP group

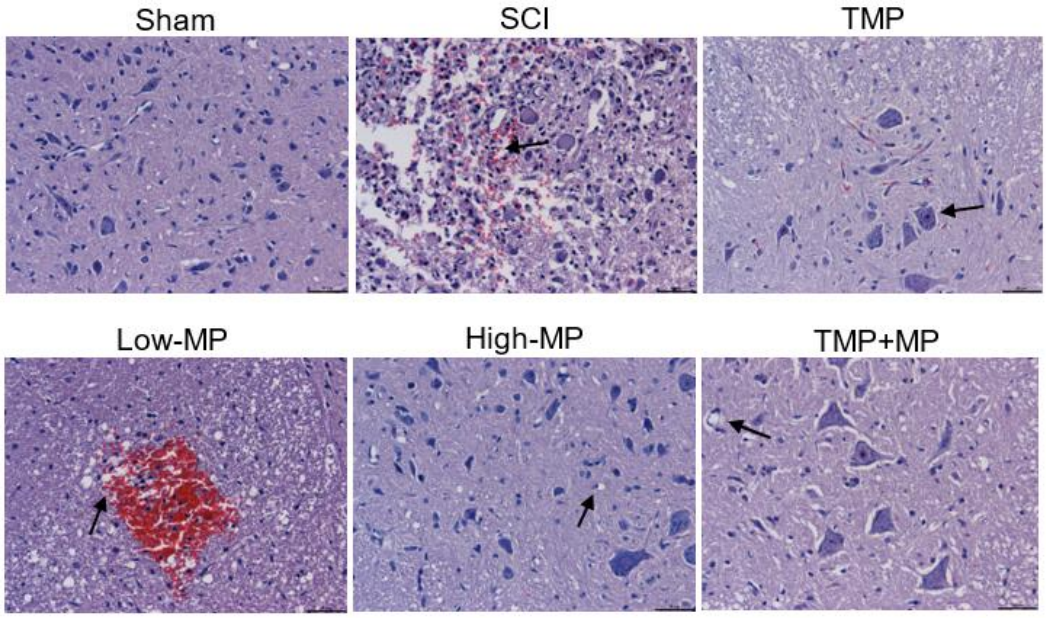

Figure 3.
Observation of SC tissue sections stained with HE in SCI rats from each group (200×). Black arrows indicate the lesion sites

Evaluation of neuronal cell apoptosis in SCI rats

TUNEL staining was used to observe the extent of apoptosis in SC tissue sections from T9-T11 segments of rats (Figure 4). TUNEL staining colour apoptotic cells brown. The apoptosis index was significantly increased in rats of the SCI group compared to the Sham group ($p < 0.05$). Compared to the SCI group, the apoptosis index decreased in the TMP and Low-MP groups, but the difference was negligible ($p > 0.05$). Conversely, rats in the High-MP and TMP + MP groups showed a significant decrease in apoptosis index compared to the SCI group ($p < 0.05$). The apoptosis index was slightly different between the High-MP and TMP + MP groups ($p > 0.05$).

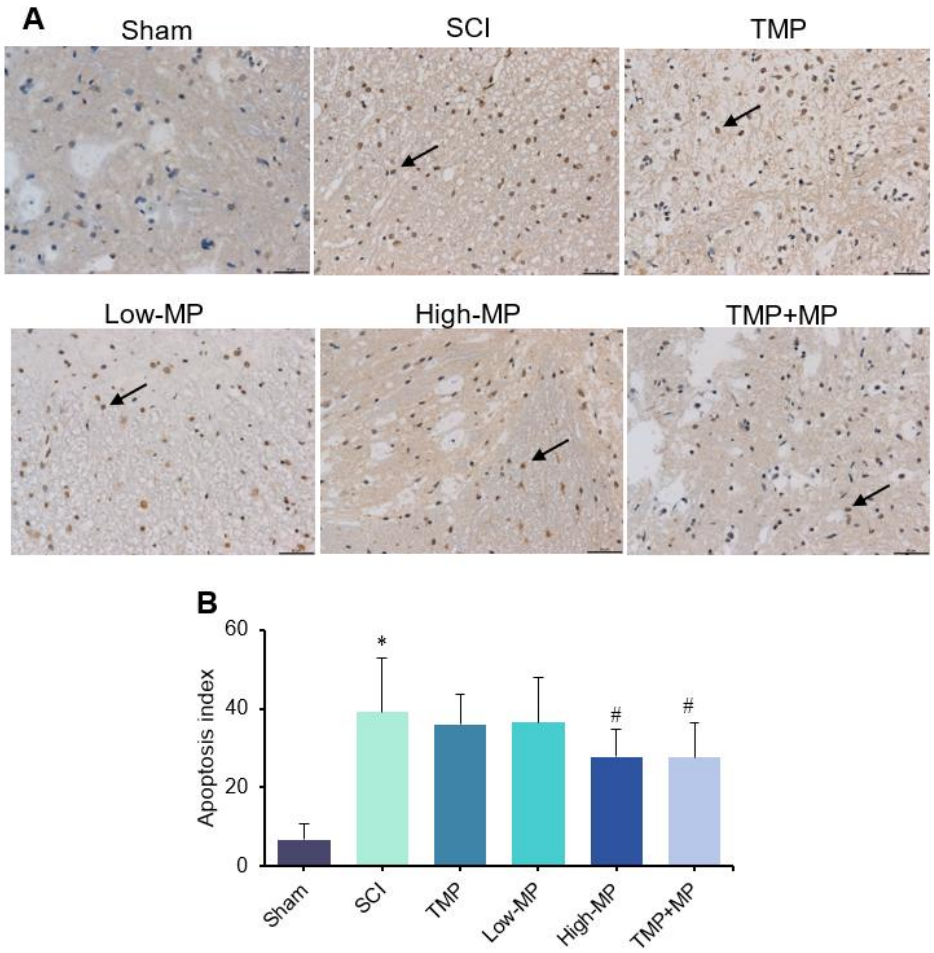

Figure 4.

The SC tissue sections of SCI rats were observed using TUNEL staining

A: TUNEL staining (200×) with black arrows indicating apoptotic cells; B: apoptosis index

*$p < 0.05$ *vs.* Sham group; #$p < 0.05$ *vs.* SCI group

Evaluation of inflammatory response in SCI rats

In Figure 5, serum IL-1β and TNF-α were significantly increased in rats in the SCI group at 1 d, 3 d and 7 d post-modelling compared to the Sham group, while the level of IL-10 was significantly decreased ($p < 0.05$). Rats in the Low-MP group showed a decrease in serum IL-1β and TNF-α at 1 d, 3 d and 7 d post-modelling compared to the SCI group, with an increase in IL-10, although the differences were not significant ($p > 0.05$). However, rats in the TMP group showed a large decrease in serum IL-1β levels at 3 d and 7 d post modelling, together with a marked increase in IL-10 levels ($p < 0.05$). Rats in the high MP group showed a remarkable decrease in serum IL-1β at 1, 3 and 7 d post-modelling, a remarkable decrease in TNF-α at 1 and 3 d and an observable increase in IL-10 at 1, 3 and 7 d ($p < 0.05$). Similarly, the TMP + MP group showed a marked decrease in serum IL-1β and TNF-α levels at 1 d, 3 d and 7 d post modelling, together with a dramatic increase in IL-10 levels at 1 d and 7 d ($p < 0.05$).

Evaluation of oxidative stress in SCI rats

Biochemical analysis was performed to assess the changes in serum oxidative stress markers in rats from each group. As shown in Figure 6, the SCI group showed a significant decrease in serum SOD levels and a large increase in serum MDA levels at 1 d, 3 d and 7 d post-modelling compared to the Sham group ($p < 0.05$). The TMP group showed a

significant decrease in MDA levels only at 1 d post-modelling compared to the SCI group ($p < 0.05$); however, the Low-MP group showed a significant increase in serum SOD levels at 1 d, 3 d and 7 d post-modelling along with a significant decrease in serum MDA levels at 1 d ($p < 0.05$). The high-MP group showed a dramatic increase in serum SOD levels at 3 and 7 d after modelling, together with a large decrease in serum MDA levels at 1 d ($p < 0.05$). The TMP + MP group showed a dramatic increase in serum SOD levels at 3 d and 7 d post-modelling, along with a large decrease in serum MDA at 3 d ($p < 0.05$).

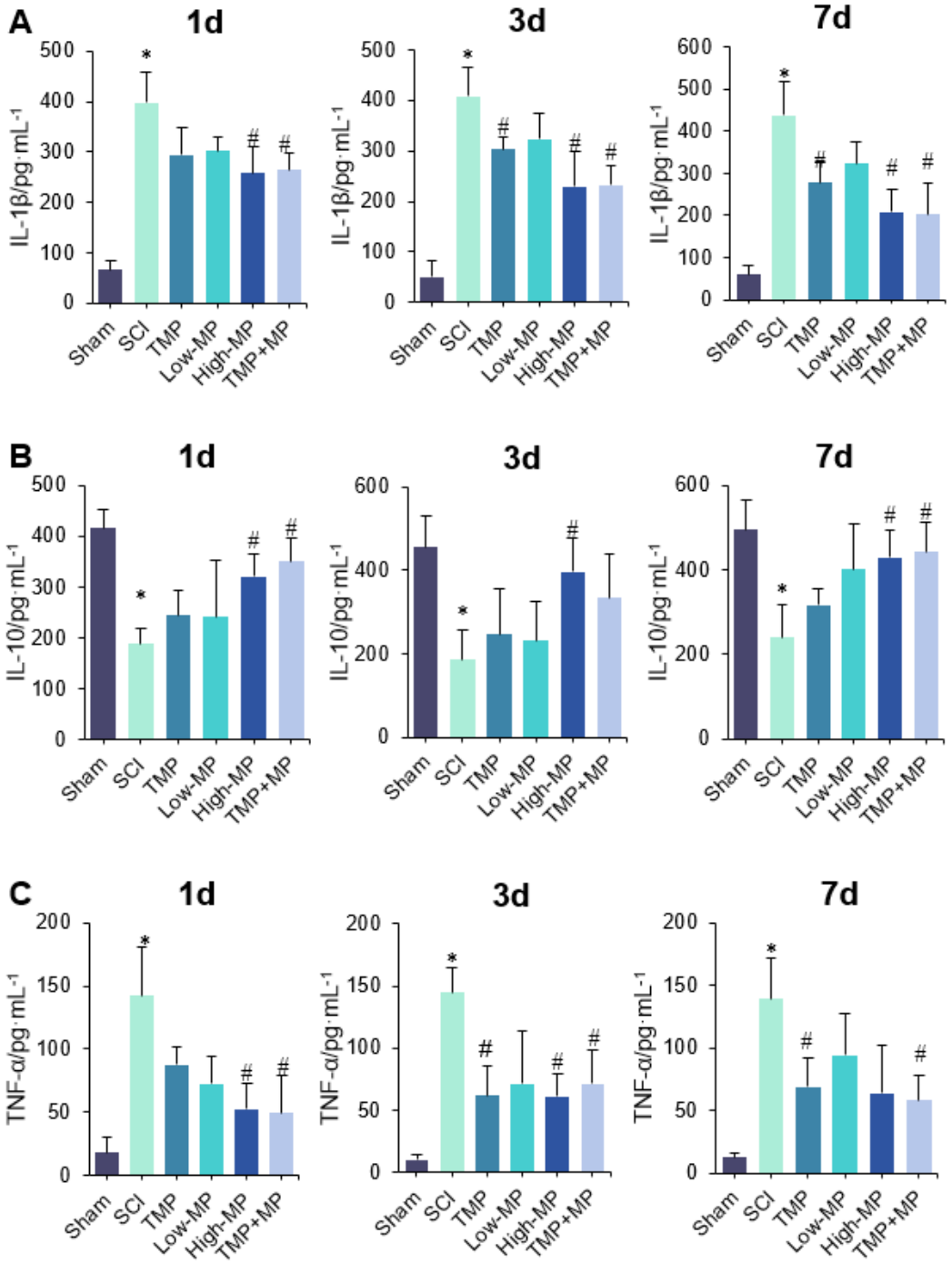

Figure 5.

Serum levels of inflammatory factors in SCI rats from each group

A: serum IL-1β at various time points; B: serum IL-10 at various time points;

C: serum TNF-α at various time points

*$p < 0.05$ *vs.* Sham group; #$p < 0.05$ *vs.* SCI group

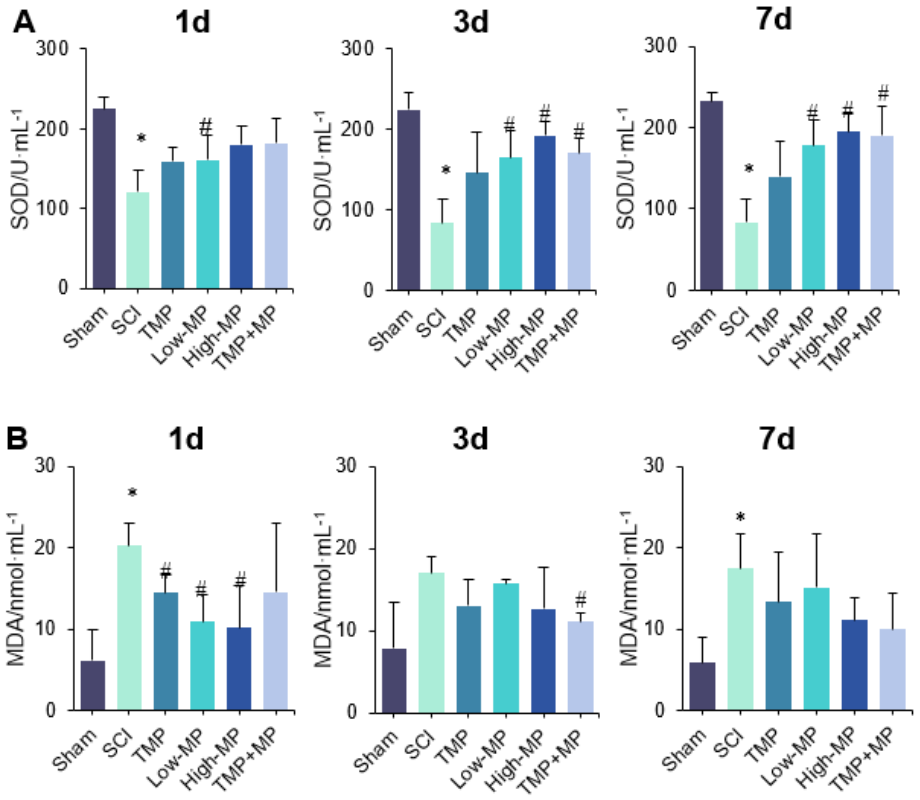

Figure 6.
Serum oxidative stress marker levels in SCI rats from each group
A: serum SOD levels at different time points are depicted; B: serum MDA levels
*$p < 0.05$ *vs.* Sham group; #$p < 0.05$ *vs.* SCI group

Evaluation of caspase-3 protein expression in SC of rats with SCI

In Figure 7, the caspase-3 protein level in the SC tissues of rats from the SCI group was significantly increased compared to the Sham group ($p < 0.05$). Conversely, caspase-3 protein levels in the SC tissues of rats from the TMP, Low-MP, High-MP and TMP + MP groups were significantly reduced compared to the SCI group ($p < 0.05$). However, the caspase-3 protein levels in the SC tissues of rats from the High-MP and TMP + MP groups did not show significant differences compared to the TMP and Low-MP groups ($p > 0.05$).

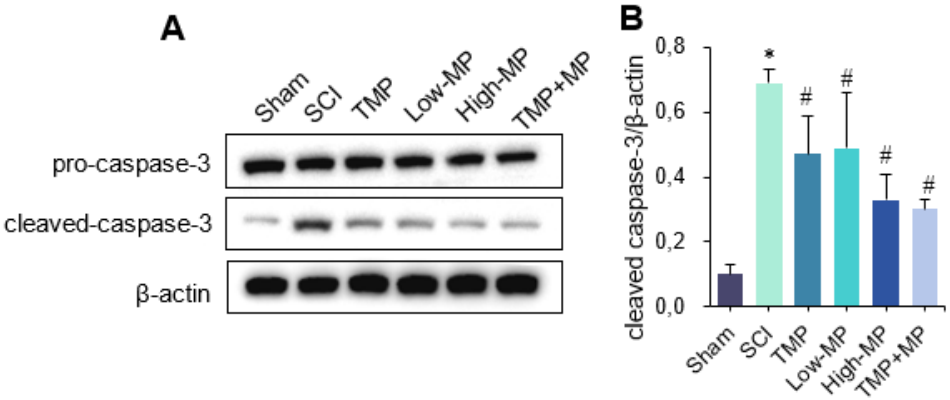

Figure 7.
Caspase-3 protein level in the SC tissues of rats with SCI
A: results of protein imprinting examination; B: cleaved caspase-3 protein level
*$p < 0.05$ *vs.* Sham group; #$p < 0.05$ *vs.* SCI group

SCI refers to the functional impairment below the damaged spinal level and its incidence has been increasing annually [17]. Currently, MP is a corticosteroid anti-inflammatory drug used clinically

for SCI treatment. It can suppress autoimmune reactions triggered by viral infections and alleviate lung cell damage caused by inflammatory factors [18]. MP effectively promotes neural functional recovery after SCI [19-21]. It was found that both low-dose and high-dose MP interventions in SCI rats resulted in notable increases in BBB scores and decreases in Reuter scores, with more pronounced improvements observed with high-dose MP treatment. This suggests that MP treatment can restore limb MF and sensory function in SCI rats. Additionally, a drastic reduction in neuronal apoptosis indices in SC tissues of SCI rats treated with different doses of MP was observed. This indicates that MP can inhibit apoptosis of neurons surrounding the lesion in SCI, which contributes to neural function recovery. MP has multifaceted neuroprotective effects, such as improving microcirculation, inhibiting lipid oxidation, and maintaining the excitability of the nervous system [22, 23]. Nevertheless, MP can lead to gastrointestinal dysfunction and induce cardiovascular complications, and long-term use may also result in osteoporosis or osteonecrosis [24]. Therefore, it is crucial to search for drugs that can effectively improve the outcomes of SCI with minimal side effects.

Chuanqiong is the dried rhizome of *Ligusticum chuanxiong*, characterised by its warm nature and spicy taste, and is known for its functions of promoting blood circulation, activating qi, dispelling wind, and eliminating dampness. It is widely adopted clinically in the therapy of diseases such as headaches and coronary heart disease [25, 26]. TMP is the main active ingredient in *Chuanqiong*. It is mainly divided into TMP hydrochloride and TMP phosphate. In recent years, synthetic TMP has been used as a new type of calcium channel blocker in clinical treatment. Feng *et al*. demonstrated that TMP could alleviate axonal and myelin damage around the infarct area in rats with cerebral infarction, reduce IL-6 expression, increase IL-10 and TGF-β expression, and reverse the transformation of microglia from M1 to M2 phenotype, thereby exerting neuroprotective effects against cerebral infarction [27]. This study found that treatment with TMP considerably promotes MF and sensory function recovery in rats with SCI, while also inhibiting apoptosis of SC neurons. This finding is in line with the results of Li *et al*. (2021), who prepared a rat model of SCI and applied a TMP-HIV reverse transcriptase activator-albumin delivery system, which promoted MF recovery in rats and reduced levels of inflammatory cytokines and oxidative stress-related factors [28]. Nevertheless, this study further demonstrated that the combination of TMP and MP resulted in a more pronounced recovery of MF and sensory functions in rats, along with a lower apoptosis index of SC neurons. This indicates that the combined use of TMP and MP has a superior neuroprotective effect after SCI.

SCI is classified into primary and secondary types, with secondary injury leading to the formation of glial scars and cystic cavities, resulting in more severe damage to neurological function [29]. The progressive pathophysiological cascade following SCI includes apoptosis signalling pathways, excitotoxicity of glutamate, and regulatory cell death [30]. Inflammatory response and glial scar formation are key factors exacerbating neurological damage and impairing MF following secondary SCI. After SCI, the lesion microenvironment promotes the release of IL-1β, IL-6 and TNF-α, which aggravate inflammation through immune-inflammatory reactions and cascading effects, thereby worsening further damage at the lesion site [31, 32]. TNF-α is the earliest responder after inflammatory reaction, promoting chemotaxis of inflammatory cells and inducing exacerbation of inflammation [33]. IL-1β can induce inflammatory response post-injury and accelerate the activation of inflammatory cells [34]. IL-10 functions as an anti-inflammatory cytokine. SOD and MDA are important biomolecules associated with cellular oxidative stress and free radical reactions. SOD catalyses the reduction of superoxide radicals into oxygen molecules and hydrogen peroxide, thereby protecting cells from damage caused by superoxide radicals and oxidative stress [35]. MDA is a lipid peroxide and is commonly considered a marker of lipid oxidation processes induced by free radicals [36]. Fan *et al*. demonstrated that TMP improves the microstructure of neuronal axons after SCI and inhibits IL-1β and IL-18 concentrations [37]. Li *et al*., through meta-analysis and systematic evaluation, confirmed that TMP promotes the recovery of neurological and MFs in acute SCI rats, increases SOD activity, and decreases MDA levels [38]. This study found that after combined treatment with TMP and MP, IL-1β, TNF-α and MDA levels in SCI rats were significantly reduced, while IL-10 and SOD activity were drastically increased. Additionally, the changes in cytokine status were more pronounced compared to TMP and low-dose MP alone. This suggests that the combination of TMP and MP can significantly downregulate pro-inflammatory cytokines IL-1β and TNF-α levels, upregulate anti-inflammatory cytokine IL-10 levels to inhibit the inflammatory response after SCI. Furthermore, it can also improve the oxidative stress status after SCI by regulating SOD activity and reducing MDA production.

Reducing neuronal apoptosis and improving oxidative stress are crucial after SCI. Caspase-3, a protease, is generally considered the principal effector in the process of cell apoptosis and a vital component of cytotoxic T lymphocyte-mediated cell killing mechanisms [39]. Studies have confirmed a significant increase in caspase-3 expression in lesioned SC tissue

following SCI [40]. This study found that after combined treatment with TMP and MP, the expression levels of caspase-3 protein in the SC tissue of SCI rats were significantly reduced, with lower expression levels compared to TMP and low-dose MP alone. This indicates that the combination of TMP and MP can decrease neuronal apoptosis rates after SCI by reducing the expression of the apoptosis-related protein caspase-3.

Conclusions

In summary, the combination of TMP and MP effectively promotes the recovery of MF and sensory function after acute SCI and outperforms TMP and low-dose MP alone. Its main mechanism involves alleviating post-injury inflammatory status and oxidative stress reactions to exert neuroprotective effects. To further elucidate the therapeutic mechanism of TMP combined with MP in treating SCI, future research should focus on signal transduction pathways.

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Conflict of interest

The authors declare no conflict of interest.

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