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REVIEW

Metformin's therapeutic potential in spinal cord injury: a systematic review and meta-analysis on locomotor recovery, neuropathic pain alleviation, and modulation of secondary injury mechanisms

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Abstract

Objective To evaluate metformin's efficacy in locomotion recovery, alleviating neuropathic pain, and modulating underlying molecular mechanisms in Spinal Cord Injury (SCI) rodent models through a systematic review and meta-analysis.

Methods We conducted a comprehensive literature search across Medline, Embase, Scopus, and Web of Science from inception to May 2024. We included studies that utilized rodent models of traumatic SCI treated with metformin versus untreated controls. Data on locomotor recovery, neuropathic pain, and molecular mechanisms related to secondary injury were extracted. Standardized mean differences (SMDs) were synthesized as the pooled effect sizes.

Results Twenty-three studies comprising 1,567 animals met the inclusion criteria. Metformin significantly enhanced locomotor function (SMD = 2.23, 95% CI: 1.74, 2.73, $p < 0.001$) and improved both mechanical allodynia (SMD = 1.18; 95% CI, 0.35 to 2.00; $p = 0.005$) and thermal hyperalgesia (SMD = 2.40; 95% CI, 1.65 to 3.16; $p < 0.001$). It reduces inflammation, oxidative stress, microglial activation, and astrogliosis and promotes myelination and autophagy flux via activating the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway. This resulted in decreased apoptosis and lesion size and increased tissue preservation and neuronal survival. Subgroup analyses indicated greater locomotor improvements when metformin was administered in the acute (< 3 days of injury) phase of the injury (meta-regression coefficient = 1.65; 95% CI, 0.37 to 2.93; $p = 0.011$).

Conclusion Metformin shows significant therapeutic benefits for SCI in rodent models, promoting locomotor recovery and alleviating neuropathic pain. These results underscore its translational potential for clinical SCI management.

Keywords Metformin · Spinal cord injury · Neuroprotection · Systematic review · Meta-analysis

Introduction

Spinal cord injury (SCI) represents a catastrophic condition often resulting from mechanical trauma, with profound consequences for motor and sensory functions [2, 8]. Affecting an estimated 750 individuals per million globally, SCI leads to significant physical disabilities, psychological burdens, and substantial healthcare costs [45, 59]. The primary phase involves direct mechanical trauma to the spinal cord, causing immediate damage. However, the real devastation occurs during the complex secondary processes, which involve a sequence of events, including inflammation, edema, ischemia, excitotoxicity, disruption of autophagy, and neuronal apoptosis. These secondary processes lead to permanent sensorimotor deficits, such as paralysis, loss of sensation, and impaired autonomic functions. Effective recovery

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from SCI relies on targeting these secondary injury cascades and reconstructing the damaged neural circuits [1, 6, 25, 39]. Despite advancements in surgical interventions, over 70% of SCI cases result in suboptimal recovery, underscoring the pressing need for novel therapeutic strategies aimed at modulating secondary injury mechanisms and promoting neural repair [57, 59].

Despite the diverse array of mechanisms involved in these pathological cascades, oxidative stress and adenosine monophosphate-activated protein kinase (AMPK) are considered pivotal drivers in the progression of SCI [50, 52]. Notably, the increased production of reactive oxygen species (ROS) subsequent to central nervous system (CNS) injury can activate mitogen-activated protein kinase (MAPK) or the nod-like receptor family, pyrin domain-containing three (NLRP3) inflammasome, eliciting a neuroinflammatory response and potentially causing neuronal harm through various pathways [21, 41]. Furthermore, activation of AMPK signaling has the ability to reduce ROS generation, thereby exhibiting anti-inflammatory and neuroprotective properties in the context of CNS injury [23].

Metformin is a biguanide antihyperglycemic agent approved by the Food and Drug Administration for treating type II diabetes mellitus and has garnered attention for its pleiotropic effects beyond glycemic control [22]. It exerts significant anti-inflammatory [35], anti-apoptotic [49], and anti-oxidative properties [3], which have demonstrated potential in mitigating the pathophysiological cascades of several neurological disorders, including Parkinson's disease, Huntington's disease, and ischemic brain injury[24, 33, 37, 38]. Of particular interest is its activation of the AMPK pathway, a critical modulator of cellular energy homeostasis and oxidative stress response [50, 52]. These neuroprotective properties position metformin as a promising candidate for addressing the multifaceted challenges associated with SCI.

Recent studies have explored metformin's potential novel application in SCI treatment, with numerous preclinical investigations documenting its beneficial neuroprotective effects [52, 53, 67, 69]. However, some studies have presented contrasting findings, pointing to a lack of positive outcomes [28]. The complexities surrounding optimal dosages, critical time frames for intervention, and frequency of metformin administration in SCI management present ongoing challenges. Furthermore, the comprehensive understanding of the safety profile and the pharmacological mechanisms underlying metformin's utilization in treating SCI remains limited.

While previous systematic studies [6, 62] primarily focused on locomotor recovery and neuronal counts after metformin therapy for SCI, the current study incorporates recent literature published after 2021 and also expands the scope by investigating broader outcomes, including locomotor recovery, pain alleviation, and underlying biomedical pathways such as inflammation, oxidative stress, metabolic components, lesion size, autophagy flux, and apoptosis.

Methods and materials

Study design

The protocol for this systematic review and meta-analysis was published in the PROSPERO database for systematic reviews (registration code: CRD42024521555). The present study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [36]. We formulated the study question using the PICO framework: P (patient/problem/population): rodent models of SCI; I (intervention): administration of metformin; C (comparison): control group with no treatment; O (outcome): measures included locomotion, neuropathic pain, metabolic changes, inflammation, microglial activation, myelination, astrogliosis, oxidative stress, autophagic flux, apoptosis, lesion characteristics, and neural cell survival.

Search strategy

A comprehensive literature search was conducted across Medline (via Pubmed), Scopus, Web of Science, and Embase, from inception to 5 May 2024. The keywords of search queries were selected using MeSH terms, Emtree terms, and their synonyms, in consultation with experts in the field. The detailed search strategy is provided in Supplementary Material 1. Additionally, a manual search was performed across Google Scholar, the Google search engine, and reference lists of relevant articles to ensure no relevant studies were missed.

Inclusion and exclusion criteria

We included peer-reviewed, controlled studies that assessed the effectiveness of metformin in treating SCI in rodent models compared to SCI rodents receiving placebo controls. Placebo controls included saline, vehicle, dimethyl sulfoxide, or no treatment. The studies focused on outcomes such as locomotor recovery, pain alleviation, and molecular mechanisms involved in secondary injuries. The exclusion criteria were as follows: in vitro studies, studies on non-rodent animals, genetically modified species, lack of outcome assessment, absence of a control group, non-traumatic SCI models (e.g., aortic cross-clamping), combination therapy of metformin with other treatments, reviews, abstracts, and duplicate publications.

Study selection and data extraction

Records from the databases were exported to EndNote (version 21; Thomson Reuters, Toronto, ON, Canada), and duplicates were removed. Two independent reviewers screened titles and abstracts, with disagreements resolved by discussion with a third reviewer. Full texts of potentially eligible articles were reviewed based on the inclusion and exclusion criteria. Data from selected articles were extracted into a predefined checklist, covering various parameters: study characteristics (author, publication year, design), population details (rodent species, strain, sex, age, weight), SCI induction characteristics (model of injury, injury level, severity, intervention details including timing, dosage, frequency, and administration route), and outcome measures.

Outcome measurements

In the study, a variety of outcome measures were utilized to assess the effects of the treatment comprehensively. Locomotion recovery was assessed using Basso, Beattie, and Bresnahan (BBB) [4] and Basso Mouse Scale (BMS) [5]. The scores were recorded at the final follow-up reported in each study. Pain alleviation was measured for mechanical allodynia through paw withdrawal threshold using the Von Frey test [44] and for thermal hyperalgesia via paw withdrawal latency using the hot plate test [17] . Metabolic changes were tracked by analyzing the phosphorylation ratio of AMP-activated protein kinase (p-AMPK/AMPK), a critical regulator of cellular energy homeostasis and metabolic activity [11, 23]. Apoptosis, a form of programmed cell death, was evaluated using multiple biomarkers. The pro-apoptotic protein Bax and the anti-apoptotic protein Bcl-2 were quantified to determine the balance between cell death and survival. Additionally, Beclin1 levels were measured to assess its dual role in apoptosis and autophagy [12, 60] . Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells were also quantified to directly detect DNA fragmentation, a hallmark of apoptosis [19]. Autophagy flux, a key cellular degradation and recycling process, was monitored through the levels of p62 (also known as sequestosome 1), a protein that accumulates when autophagy is impaired [13]. Inflammatory responses were analyzed by measuring pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). Elevated levels of these cytokines are indicative of a pro-inflammatory response [1, 9]. Neuroinflammation and gliosis, which contribute to secondary injury after trauma, were quantified by evaluating microglial activation and astrogliosis. Microglial activation was measured by counting cells positive for ionized calcium-binding adapter molecule 1 (Iba1), while astrogliosis was assessed using glial fibrillary acidic protein (GFAP)-positive cells, reflecting the extent of reactive astrocyte proliferation [30, 31]. Myelination, essential for axonal conduction and neuronal communication, was determined by analyzing myelin basic protein (MBP) levels and MBP + area. This provided insights into the preservation or regeneration of myelin sheaths [58]. Oxidative stress, a key driver of cellular damage, was assessed through the levels of malondialdehyde (MDA), an indicator of lipid peroxidation, and superoxide dismutase (SOD), an antioxidant enzyme that mitigates oxidative damage [42, 60]. Lesion characteristics were detailed by measuring lesion size, the extent of spared tissue, and neuronal cell survival. Neuronal nuclei (NeuN)-positive cells and Nissl-positive cells were quantified to evaluate neuronal integrity and survival within the injured area [51]. These metrics collectively provide a comprehensive view of structural and functional outcomes in the context of the studied conditions.

Risk of bias assessment and certainty of evidence

The quality control of the included studies was assessed using the Systematic Review Centre for Laboratory Animal Experimentation's (SYRCLE's) risk of bias tool [20]. To evaluate the certainty of evidence for each outcome, we applied the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) framework [15]. Any conflicts or disagreements were resolved by consulting a third researcher.

Statistical analyses

The statistical analyses were conducted using Stata 17.0 (StataCorp LLC, College Station, TX, USA). We synthesized study results and reported pooled effect sizes as standardized mean differences (SMD) with a 95% confidence interval (CI). Due to methodological and clinical heterogeneity among included original studies, a random-effects model was used. Statistical heterogeneity between studies was assessed using I^2 and chi-square tests. In the case of heterogeneity, subgroup analyses and meta-regression were performed to identify the potential sources. Meta-analysis was only conducted if data were reported by at least three separate experiments. Outlier studies were identified using Galbraith's plot and further evaluated with the input of an expert neurosurgeon who has over 10 years of experience in the field of SCI. Subgroup analyses and meta-regression were performed for outcomes with at least ten experiments to investigate the effects of different factors, including animal species, models of SCI, SCI severity, intervals from SCI to treatment, metformin dosages and frequency, and injection routes. Metformin doses were categorized as low (≤ 50 mg/kg) and high (> 50 mg/kg), while the injury to treatment interval was organized into acute (< 3 days) and subacute

(3–7 days) according to studies by Zhang et al.(2022) [62], Song et al. [46] [46]. For outcomes with at least ten experiments, publication bias assessment was visualized by Funnel Plot and tested by the modified Egger's test suggested by Doleman et al. (2021) [7].

Results

Study selection and characteristics of the included studies

The systematic search of electronic databases yielded 1419 non-duplicated studies. After screening titles and abstracts, 28 full-text articles were reviewed in detail. Ultimately, 23 studies were included in the meta-analysis [1, 10, 14, 16, 26, 29, 46, 51, 52, 58, 60, 63–65, 68]. The PRISMA flowchart for study selection process is depicted in Fig. 1.

Characteristics of the included studies are summarized in Table 1. The studies included data from 1,567 animals (770 in the control group and 797 in the treatment group). Among these, 16 studies involved rats (14 Sprague–Dawley and 2 Wistar), and 7 used C57BL/6 J mice. Regarding the model of SCI, 13 studies employed the contusion model, and 10 studies used the compression model. None of the included studies utilized combination models. The injury level in all included studies was thoracic. As for injury severity, it was classified as severe in 16 studies and moderate in 7 studies. Initial doses of metformin varied from 2 mg/kg to 516 mg/kg, and cumulative doses ranged from 10 mg/kg to 6400 mg/kg. The intraperitoneal route was most common (18 studies), followed by intravenous (3 studies) and intrathecal (1 study); one study did not specify the administration route. The majority of studies treated animals in the acute phase of injury (19 studies), while metformin was used in the subacute phase in 2 studies and in both acute and subacute phases in another two studies. Five studies used a single dose of metformin, whereas 18 studies administered 3 to 53 repeated doses. Among the mechanisms studied in relation to metformin's effects, the most frequently examined were inflammation markers in 12 studies, apoptotic markers in 10 studies, oxidative stress markers in 7 studies, and autophagy markers in 6 studies.

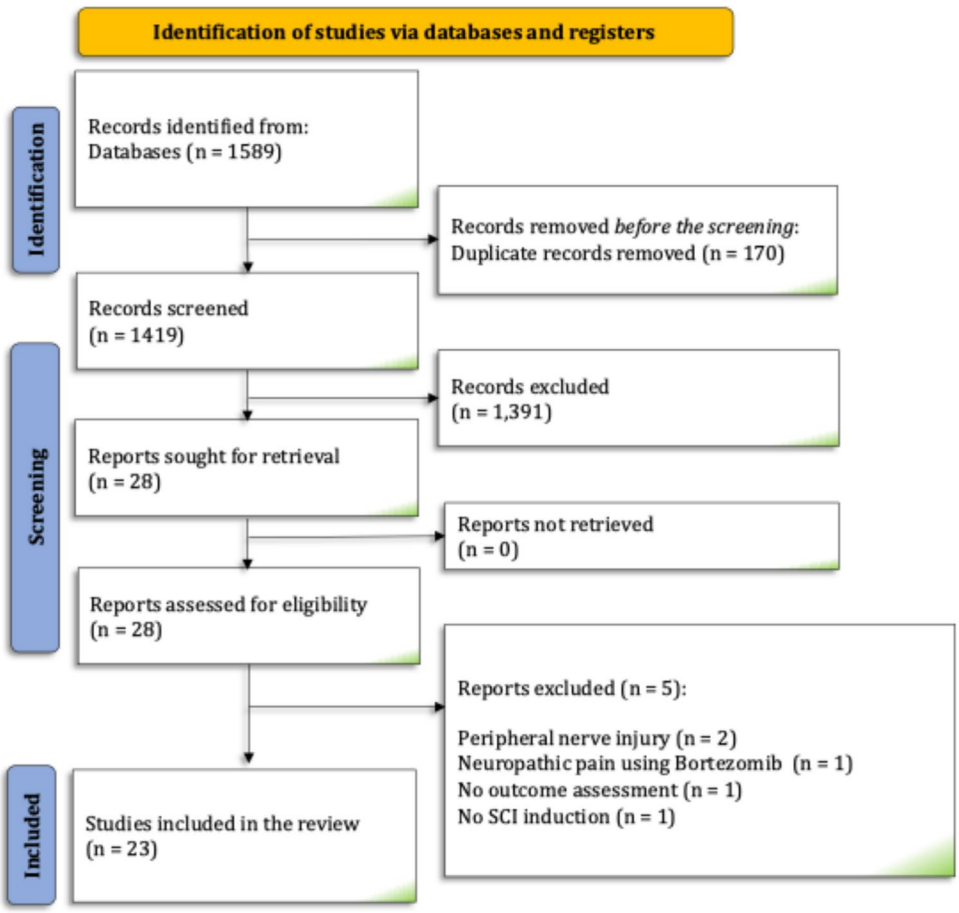

Fig. 1 PRISMA (preferred reporting items for systematic reviews and meta-analyses) flow diagram of the screening process

Table 1 Summary characteristics of the included studies

Author, year, country	Gender, strain, species, weight (gram)	Number of controls/ treated	SCI model, induction method, severity	Initial dose (mg/kg), total dose (mg/kg), route of administration	Treatment timing, Treatment frequency	Mechanism studied
Afshar, 2018, Iran	Male, Rat, SD, 240–260	7/7	Compression, Aneurysmal clip, Severe	10/50/100, 10/50/100, IP	Immediate, Single dose	Inhibition of Inflammation
Astaraky, 2022, Iran	Male/Female, Rat, Wistar, 230–250	3–7/3–7	Compression, Aneurysmal clip Severe	516/1032/2064,516/1032/2064, IT	30 min post surgery, Single dose	Inhibition of Oxidative Stress
Gilbert,2022, Canada	Male/Female, Mice, C57BL/6 J, 20–25	5/5	Contusion, Bend needle insersion Severe	200,1400, IP	Immediate/ 7 days post SCI, 7/ 14 doses	Inhibition of Microglial Activation and Activation of Resident Precursor Cells
Guo, 2018, China	Female, Rat, SD, 200–240	4–5/4–5	Contusion, Weight drop, Severe	10,30, IP	Immediate, 3 doses	Regulation of Autophagy and Inhibition of Apoptosis
Huange, 2023, China	Male, Mice, C57BL/6 J, 18–20	4–8/ 4–8	Contusion, Impactor, Moderate	100, 4200, IP	Immediate, 42 doses	Promotion of Remyelination
Li, 2022, China	Female, Rat, SD, 180–220	3–5/3–5	Contusion, Weight drop, Severe	25, 175, IP	6 h post injury, 7 doses	Inhibition of Inflammation and Apoptosis
Lin, 2015, Taiwan	Male, Rat, Wistar, NM	6/6	Compression, Aneurysmal clip, Severe	320,6400, IV	7 days post SCI, 20 doses	Regulation of Autophagy
Liu,2023, China	NM, Mice, C57BL/6 J, NM	3/3	Contusion, Impactor, Moderate	NM, NM, NM	Immediate, 7 doses	Inhibition of Oxidative Stress, Inflammation, and Apoptosis
Wang, 2016, China	Female, Rat, SD, 180–220	4–6/4–6	Contusion, Impactor, Moderate	100/200, 300/2800, IP	Immediate/ 2 weeks before SCI, 3/14 doses	Regulation of Autophagy and Inhibition of Inflammation and Apoptosis
Wang, 2018, China	Male, Rat, SD, 200–220	5/5	Compression, Bulldog clamp, Severe	50,50, IP	1 day post surgery, Single dose	Regulation of Autophagy
Wang, 2020, China	Female, Rat, SD, 220–250	5–10/5–10	Compression, Vascular clip, Moderate	50,700, IP	Immediate, 14 doses	Inhibition of Oxidative Stress and Apoptosis and Stabilizing Microtubules
Wang, 2022, China	Male, Rat, SD, 220–250	5–10/5–10	Compression, Aneurysmal clip Moderate	200, 2800, IP	Immediate, 14 doses	Inhibition of Oxidative Stress, Inflammation, and Ferroptosis
Wang, 2023, China	Male, Rat, SD, 220–250	5–6/5–6	Compression, Aneurysmal clip, Severe	200, 2800, IP	30 min post surgery, 14 doses	Inhibition of Inflammation and Ferroptosis
Wu, 2021, China	Female, Rat, SD, 220–250	5–8/5–8	Compression, Vascular clip, Severe	50,1400, IP	Immediate, 28 doses	Regulation of Autophagy and Inhibition of Inflammation, Microglial Activation, and Apoptosis
Ye-Song,2020, China	Male/Female, Mice, C57BL/6 J, 20–25	5/7–8	Contusion, Impactor, Moderate	100,700, IP	Immediate/ 3 days post SCI, 7 doses	Inhibition of Microglial and Astrocyte Activation and Neutrophil Infiltration

Table 1 (continued)

Author, year, country	Gender, strain, species, weight (gram)	Number of controls/ treated	SCI model, induction method, severity	Initial dose (mg/kg), total dose (mg/kg), route of administration	Treatment timing, Treatment frequency	Mechanism studied
Yu, 2022, China	Male/Female, Mice, C57BL/6 J, 21–25	6–8/6–8	Contusion, Impactor, Severe	50,50, IP	Immediate, Single dose	Inhibition of Oxidative Stress, Inflammation, and Apoptosis
Yuan,2022, China	Female, Rat, SD, 180–220	3/3	Contusion, Impactor, Severe	50,100, IP	Immediate/ 1 days post SCI, 2 doses	Inhibition of Inflammation and Pyroptosis
Yuan,2023, China	Female, Rat, SD, 200–250	3–7/3–7	Contusion, Weight drop, Severe	2,10, IV	Immediate, 5 doses	Inhibition of Oxidative Stress, Inflammation, Apoptosis, and Activation of Precursor Cells
Zhang, 2016, China	Female, Rat, SD, 220–250	5/5	Compression, Vascular clip, Severe	50,700, IP	Immediate, 14 doses	Regulation of Autophagy and Inhibition of Apoptosis
Zhang, 2017, China	Female, Rat, SD, 220–250	5/5	Compression, Vascular clip, Severe	50,700, IP	Immediate, 14 doses	Inhibition of Neutrophil Infiltration and Blood–Brain Barrier Degeneration
Zhang, 2020, China	Male, Rat, SD, 180–200	3–6/ 3–6	Contusion, Impactor, Severe	50,700, IP	Immediate, 14 doses	Inhibition of Inflammation and Apoptosis
Zhao,2022, China	Male, Mice, C57BL/6 J, 32–38	3–6/ 3–6	Contusion, Weight drop, Moderate	100,5600, IP	3 days post SCI, 53 doses	Promotion of Angiogenesis
Zhu,2023, China	Male/Female, Mice, C57BL/6 J, 20–30	3/3	Contusion, Impactor, Severe	NM, IV	Immediate, Single dose	Inhibition of Oxidative Stress, Inflammation, and Microglial Activation

IP Intraperitoneal; *IT* Intrathecal; *IV* Intravenous; *NM* Not mentioned; *SCI* Spinal cord injury; *SD* Sprague–Dawley; *SNL* Spinal nerve ligation

Meta-analysis

a) Locomotion

The meta-analysis for the evaluation of metformin efficacy on locomotion recovery incorporated 26 separate analyses. Pooled data analysis demonstrated that metformin significantly improves locomotion recovery after SCI (SMD, 2.23; 95% CI, 1.74 to 2.73; $p < 0.001$; $I^2 = 69.09\%$) (Fig. 2). Subgroup analyses and meta-regressions were conducted to identify sources of heterogeneity. The improvement in locomotion recovery was significant in almost all subgroups, except for animals treated in the sub-acute phase of SCI ($p = 0.137$) and animals receiving intravenous metformin administration ($p = 0.113$), which were each investigated in only three distinct experiments. Meta-regressions demonstrated that the extent of improvement is significantly greater in animals treated in the acute phase of SCI compared to the sub-acute phase (meta-regression coefficient, 1.65; 95% CI, 0.37 to 2.93; $p = 0.011$). However, no significant differences were observed between rodent species (rats vs. mice), SCI models, injury severity, treatment dosage, treatment frequency, or route of administration. (Table 2). Additionally, the evaluation of evidence for metformin's effect on locomotion recovery using the GRADE framework indicated a high level of evidence (Table 3). No evidence of publication bias was observed for locomotion recovery (Supplementary Fig. 1).

b) Neuropathic pain

The meta-analysis assessing metformin's effectiveness on pain included two categories, mechanical allodynia and thermal hyperalgesia, with seven and six experiments, respectively. Analysis of pooled data revealed that metformin significantly alleviated mechanical pain, as indicated by an increased withdrawal threshold (SMD, 1.18; 95% CI, 0.35 to 2.00; $p = 0.005$; $I^2 = 73.02\%$) and thermal hyperalgesia, as shown by increased withdrawal latency (SMD, 2.40; 95% CI, 1.65 to 3.16; $p < 0.001$; $I^2 = 46.57\%$) (Fig. 3). The GRADE framework indicated high evidence for thermal and moderate for mechanical pain alleviation (Table 3).

c) Cell metabolism

The meta-analysis assessing metformin's effect on Adenosine Monophosphate-Activated Protein Kinase (AMPK) activation included six analyses. Pooled data demonstrated metformin significantly increased p-AMPK/AMPK ratio (SMD, 6.70; 95% CI, 3.92 to 9.49; $p < 0.001$; $I^2 = 74.57\%$) (Fig. 4A). The GRADE framework indicated moderate evidence for AMPK activation (Table 3).

d) Inflammation

The meta-analysis assessed metformin's effect on inflammatory indexes for three pro-inflammatory cytokines: TNF-α (5 experiments), IL-6 (4 experiments), and IL-1β (5 experiments). Pooled data showed metformin significantly reduced TNF-α (SMD, −2.78; 95% CI, −4.66 to −0.90; $p < 0.001$; I2 = 79.89%), IL-6 (SMD, −5.01; 95% CI, −6.87 to −3.14; $p < 0.001$; I2 = 69.03%), and IL1β (SMD, −3.53; 95% CI, −5.80 to −1.26; $p < 0.001$; I2 = 83.30%) (Fig. 4B). The GRADE framework indicated moderate evidence for inhibiting inflammatory indexes (Table 3).

e) Microglial activation

The meta-analysis assessed metformin's effect on microglial activation by evaluating Iba1-positive (Iba1 +) area (5 experiments). Pooled data showed metformin significantly reduced microglial activation, decreasing Iba1 + area (SMD, −0.52; 95% CI, −1.03 to −0.01; $p = 0.046$; I2 = 0.00%) (Fig. 4C). The GRADE framework indicated high evidence for inhibiting microglial activation (Table 3).

f) Myelination

The meta-analysis evaluated metformin's effect on myelination by assessing the MBP + area (3 experiments). Pooled data showed metformin significantly enhanced myelination, demonstrated by an increased MBP + area (SMD = 4.02; 95% CI = 0.57 to 7.48; $p = 0.022$; $I^2 = 78.63\%$) (Fig. 4D), with moderate evidence based on the GRADE framework (Table 3).

g) Astrogliosis

The meta-analysis evaluated metformin's effect on astrogliosis by quantifying the GFAP + area (5 experiments). Pooled data revealed metformin significantly reduced astrogliosis, demonstrated by a decreased GFAP + area (SMD = −1.85; 95% CI = −3.70 to −0.01; $p = 0.049$; $I^2 = 85.84\%$) (Fig. 4E). The GRADE framework indicated moderate evidence for inhibiting astrogliosis (Table 3).

h) Oxidative stress

The meta-analysis assessed metformin's effect on oxidative stress indexes, including SOD (5 experiments) and MDA (5 experiments). Pooled data showed metformin significantly

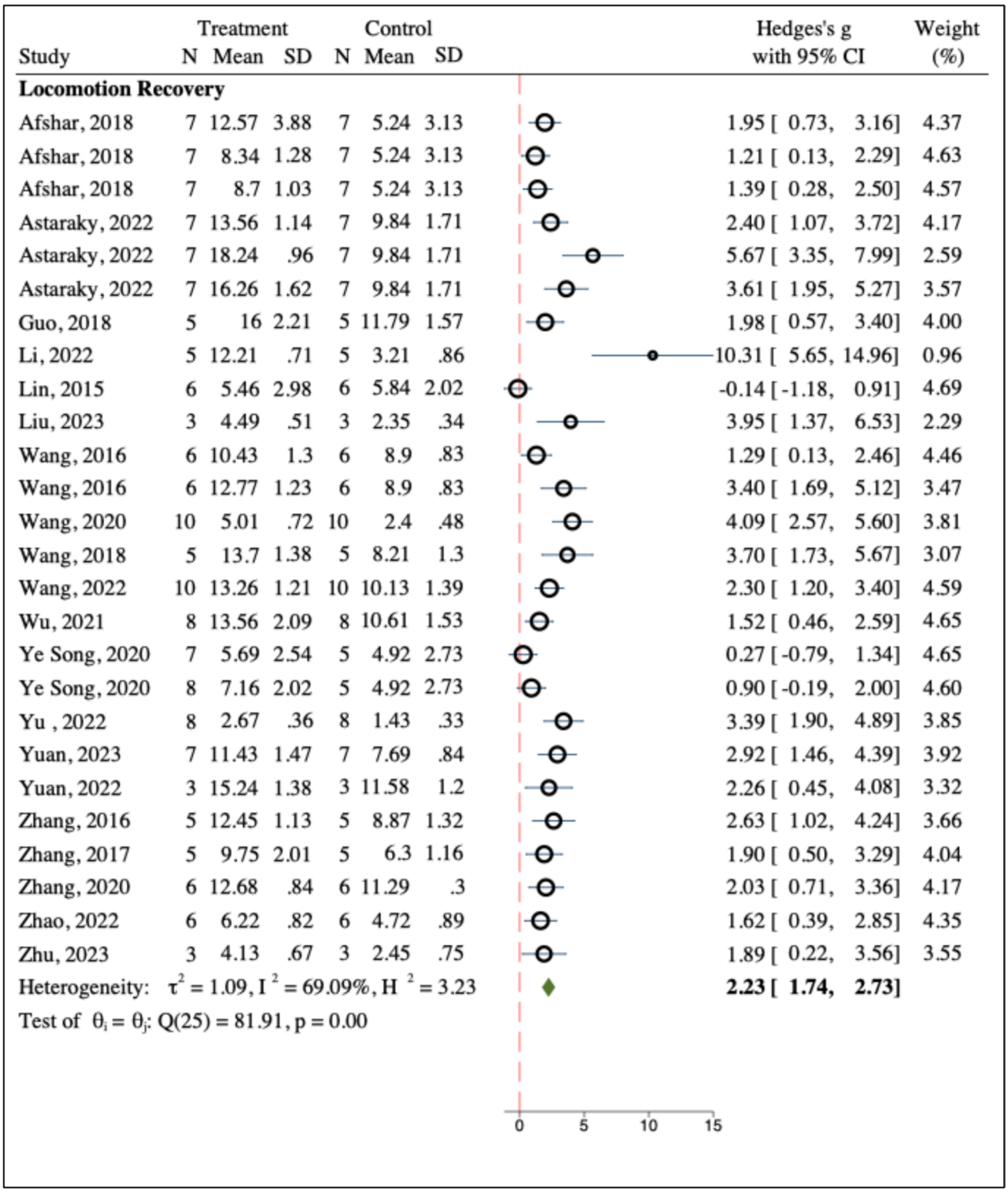

Fig. 2 The forest plot for the efficacy of metformin in locomotion recovery. SD, standard deviation; CI, confidence interval

reduced oxidative stress by decreasing MDA (SMD, −2.26; 95% CI, −3.48 to −1.05; $p < 0.001$; I2 = 59.53%) and increasing SOD (SMD, 2.42; 95% CI, 0.52 to 4.31; $p < 0.001$; I2 = 82.86%) (Fig. 5A). The GRADE framework indicated moderate evidence for inhibiting oxidative stress indexes (Table 3).

Table 2 Subgroup analysis and meta-regressions for variables influencing locomotion recovery in animal models treated with metformin

Subgroup	Number of experiments	Subgropup analysis			Meta-regression	
		SMD [95%CI]	*P* value	I^2 (%)	Coefficient [95%CI]	*P* value
Animal						
Rat	21	2.42 [1.86 to 2.98]	< 0.0001	68.14	Reference	
Mice	5	1.52 [0.50 to 2.55]	0.003	68.57	−0.88 [−2.09 to 0.33]	0.153
SCI model						
Contusion	13	2.17 [1.48 to 2.85]	< 0.0001	63.15	Reference	
Compression	13	2.30 [1.56 to 3.04]	< 0.0001	74.96	0.08 [−0.93 to 1.10]	0.873
Injury severity *						
Moderate	6	2.01 [0.81 to 3.21]	0.001	80.06	Reference	
Severe	17	2.26 [1.65 to 2.87]	< 0.0001	66.40	0.31 [−0.92 to 1.55]	0.620
Treatment timing						
Acute phase	23	2.43 [1.94 to 2.92]	< 0.0001	61.31	Reference	
Sub-acute phase	3	0.76 [−0.24 to 1.75]	0.137	57.83	−1.65 [−2.93 to −0.37]	**0.011**
Metformin dose *						
≤ 50 mg/kg	12	2.44 [1.86 to 3.01]	< 0.0001	44.02	Reference	
> 50 mg/kg	11	1.87 [1.00 to 2.74]	< 0.0001	80.82	−0.72 [−1.78 to 0.33]	0.179
Treatment frequency						
Single injection	10	2.53 [1.80 to 3.26]	< 0.0001	58.61	Reference	
Repeated injection	16	2.05 [1.39 to 2.71]	< 0.0001	72.95	−0.53 [−1.55 to 0.48]	0.305
Route of administration						
IP	23	2.32 [1.82 to 2.88]	< 0.0001	65.12	Reference	
IV	3	1.49 [−0.35 to 3.33]	0.113	81.37	−0.90 [−2.42 to 0.61]	0.244

CI Confidence interval; *IP* Intraperitoneal; *IV* Intravenous; *SCI* Spinal cord injury; *SMD* Standardized mean difference

* The discrepancy between the cumulative number of studies across each subgroup and the total number of studies included in the meta-analysis stems from missing or incomplete information

i) Autophagic flux

The meta-analysis assessed metformin's effect on autophagic indexes, p62 (3 experiments). Pooled data showed metformin significantly enhanced autophagy by reducing p62 (SMD, −2.67; 95% CI, −3.70 to −1.64; $p < 0.001$; I2 = 0.00%) (Fig. 5B). The GRADE framework indicated high evidence for p62, suggesting autophagy induction (Table 3).

j) Apoptosis

The meta-analysis evaluated metformin's effect on apoptosis indexes: pro-apoptotic (Bax—4 experiments), anti-apoptotic (Bcl-2—4 experiments, Beclin1—3 experiments), and TUNEL-positive cells (5 experiments). Pooled data showed metformin significantly decreased apoptosis, demonstrated by reduced Bax (SMD = −3.62; 95% CI = −5.12 to −2.11; $p < 0.001$; $I^2 = 52.96\%$), and TUNEL-positive cells (SMD = −4.55; 95% CI = −5.78 to −3.31; $p < 0.001$; $I^2 = 0.00\%$), and increased Bcl-2 (SMD = 3.42; 95% CI = 2.01 to 4.84; $p < 0.001$; $I^2 = 52.17\%$) and Beclin-1 (SMD = 1.70; 95% CI = 0.55 to 2.86; $p = 0.004$; $I^2 = 44.44\%$) (Fig. 5C). The GRADE framework indicated high evidence for Beclin1 and TUNEL-positive neurons and moderate evidence for Bax and Bcl-2, suggesting inhibition of apoptosis (Table 3).

k) Lesion and sparring tissue

The meta-analysis evaluated metformin's effect on lesion size (6 experiments), spared tissue size (4 experiments), NeuN (4 experiments), and Nissl-positive cells (5 experiments). Pooled data showed metformin significantly reduced lesion size (SMD = −2.24; 95% CI = −2.88 to −1.60; $p < 0.001$; $I^2 = 0.00\%$) and increased spared tissue (SMD = 2.56; 95% CI = 1.38 to 3.75; $p < 0.001$; $I^2 = 51.43\%$), NeuN-positive cells (SMD = 2.11; 95% CI = 0.37 to 3.85; $p = 0.018$; $I^2 = 82.88\%$), and Nissl-positive cells (SMD = 3.29; 95% CI = 1.12 to 5.46; $p = 0.003$; $I^2 = 81.60\%$) (Fig. 6). GRADE indicated high evidence for reduced lesion size and moderate evidence for other outcomes (Table 3).

Table 3 The certainty of evidence regarding the effect of metformin on spinal cord injury

Outcome	Number of experiments	Risk of bias	Imprecision	Inconsistency (I2 range)	Indirectness	Publication bias	Level of evidence
Locomotion Recovery	26	Not serious	No serious imprecision	No serious inconsistency*	No serious indirectness	No publication bias	High
Pain							
Mechanical pain	8	Not serious	No serious imprecision	Serious	No serious indirectness	NA	Moderate
Thermal pain	7	Not serious	No serious imprecision	No serious imprecision	No serious indirectness	NA	High
Cellular metabolic							
AMPK	6	Not serious	No serious imprecision	Serious	No serious indirectness	NA	Moderate
Inflammation							
TNF-α	5	Not serious	No serious imprecision	Serious	No serious indirectness	NA	Moderate
IL-1β	6	Not serious	No serious imprecision	Serious	No serious indirectness	NA	Moderate
IL-6	5	Not serious	No serious imprecision	Serious	No serious indirectness	NA	Moderate
Microglial							
Iba1 + area	5	Not serious	No serious imprecision	No serious imprecision	No serious indirectness	NA	High
Oxidative stress							
MDA	5	Not serious	No serious imprecision	Serious	No serious indirectness	NA	Moderate
SOD	5	Not serious	No serious imprecision	Serious	No serious indirectness	NA	Moderate
Astrogliosis							
GFAP + area	5	Not serious	No serious imprecision	Serious	No serious indirectness	NA	Moderate
Myelination							
MBP + area	3	Not serious	No serious imprecision	Serious	No serious indirectness	NA	Moderate
Autophagic							
P62	4	Not serious	No serious imprecision	No serious imprecision	No serious indirectness	NA	High
Apoptosis							
Bax	5	Not serious	No serious imprecision	No serious imprecision	No serious indirectness	NA	Moderate
Bcl-2	5	Not serious	No serious imprecision	No serious imprecision	No serious indirectness	NA	Moderate
Beclin1	4	Not serious	No serious imprecision	No serious imprecision	No serious indirectness	NA	High
TUNEL + neurons	5	Not serious	No serious imprecision	No serious imprecision	No serious indirectness	NA	High
Lesion							
Lesion size	6	Not serious	No serious imprecision	No serious imprecision	No serious indirectness	NA	High
Speard Tissue Size	4	Not serious	No serious imprecision	Serious	No serious indirectness	NA	Moderate
NeuN possitive cells	4	Not serious	No serious imprecision	Serious	No serious indirectness	NA	Moderate
Nissl positive cells	5	Not serious	No serious imprecision	Serious	No serious indirectness	NA	Moderate

AMPK Adenosine monophosphate-activated protein kinase; *Bax* Bcl-2-Associated X protein; *Bcl-2* B-cell lymphoma 2; *GFAP* Glial fibrillary acidic protein; *Iba1* Ionized calcium-binding adapter molecule 1; *IL-1β* Interleukin-1 beta; *IL-6* Interleukin-6; *LC3* Microtubule-associated protein 1 light chain 3; *MBP* Myelin basic protein; *MDA* Malondialdehyde; *NeuN* Neuronal nuclei; *Nissl* Nissl substance (Rough Endoplasmic Reticulum); *P62* Sequestosome 1/SQSTM1; *SOD* Superoxide dismutase; *TNF-α* Tumor necrosis factor-alpha; *TUNEL* Terminal deoxynucleotidyl transferase dUTP nick end labeling

*There is no serious inconsistency since the sources of heterogeneity were identified

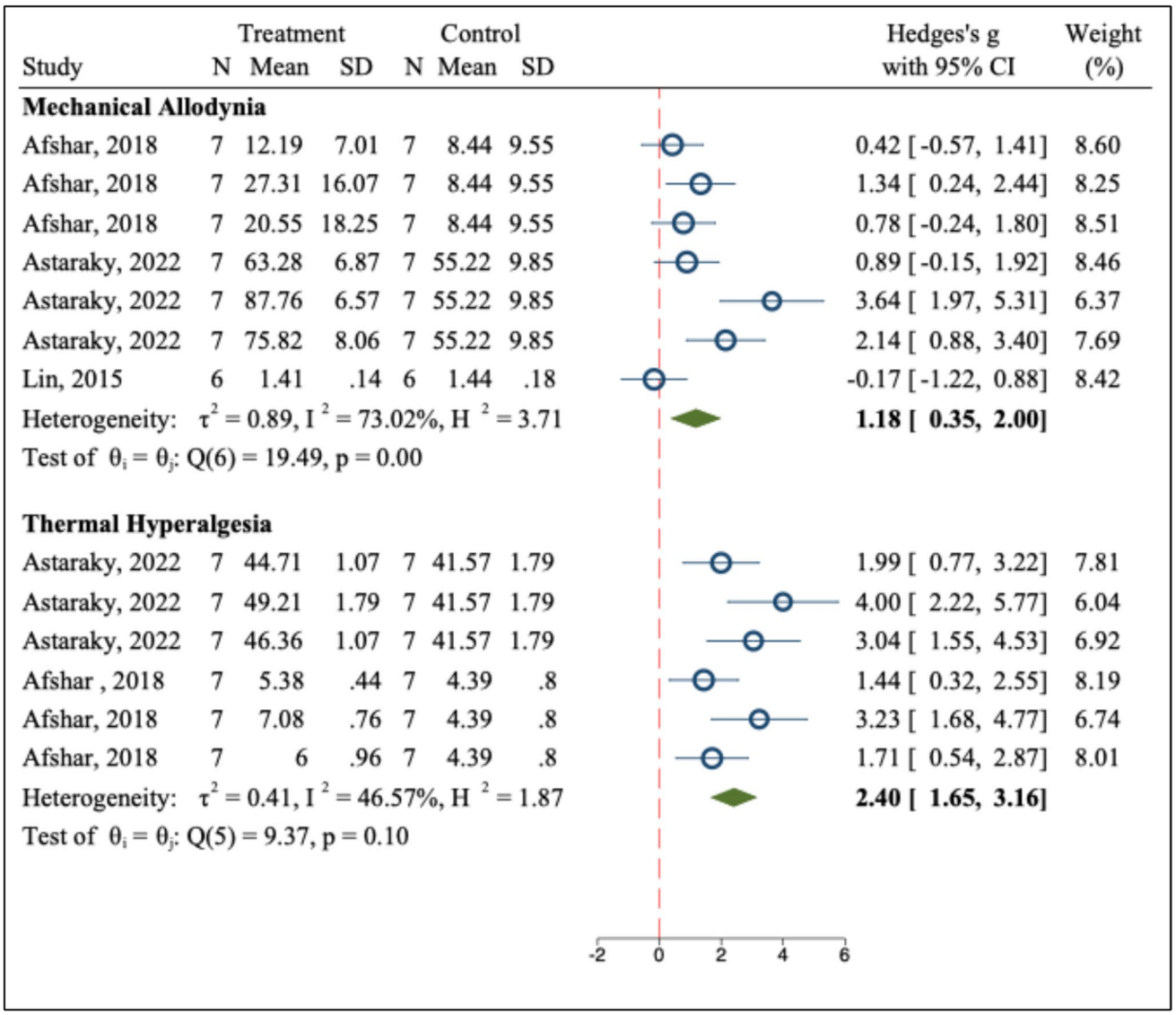

Fig. 3 The forest plot for the efficacy of metformin in pain alleviation. SD, standard deviation; CI, confidence interval

Risk of bias

The allocation generation was appropriately generated and implemented in 11 of the studies. However, none of the studies transparently disclosed how allocation concealment was managed during the experiments. Additionally, none of the studies explicitly addressed caregiver blinding. On the other hand, outcome assessor blinding was reported in 16 studies, and random outcome assessment was mentioned in nine of the studies. It remains unclear whether a random selection process was employed for outcome assessment in any of the studies. There was no evidence of incomplete outcome data or other factors that might introduce bias (Table 4).

Discussion

This systematic review and meta-analysis comprehensively investigated the neuroprotective effects of metformin, a widely used antidiabetic drug, in rodent models of SCI. After conducting a thorough systematic search, 23 studies involving a total of 1,567 animals were included in the analysis. The meta-analysis showed that metformin substantially enhanced locomotor recovery and alleviated mechanical allodynia and thermal hyperalgesia. Furthermore, metformin modulated multiple underlying pathways implicated in secondary injury cascades. Specifically, it exerted anti-inflammatory and antioxidant effects, inhibited microglial activation and astrogliosis, promoted myelination and autophagy

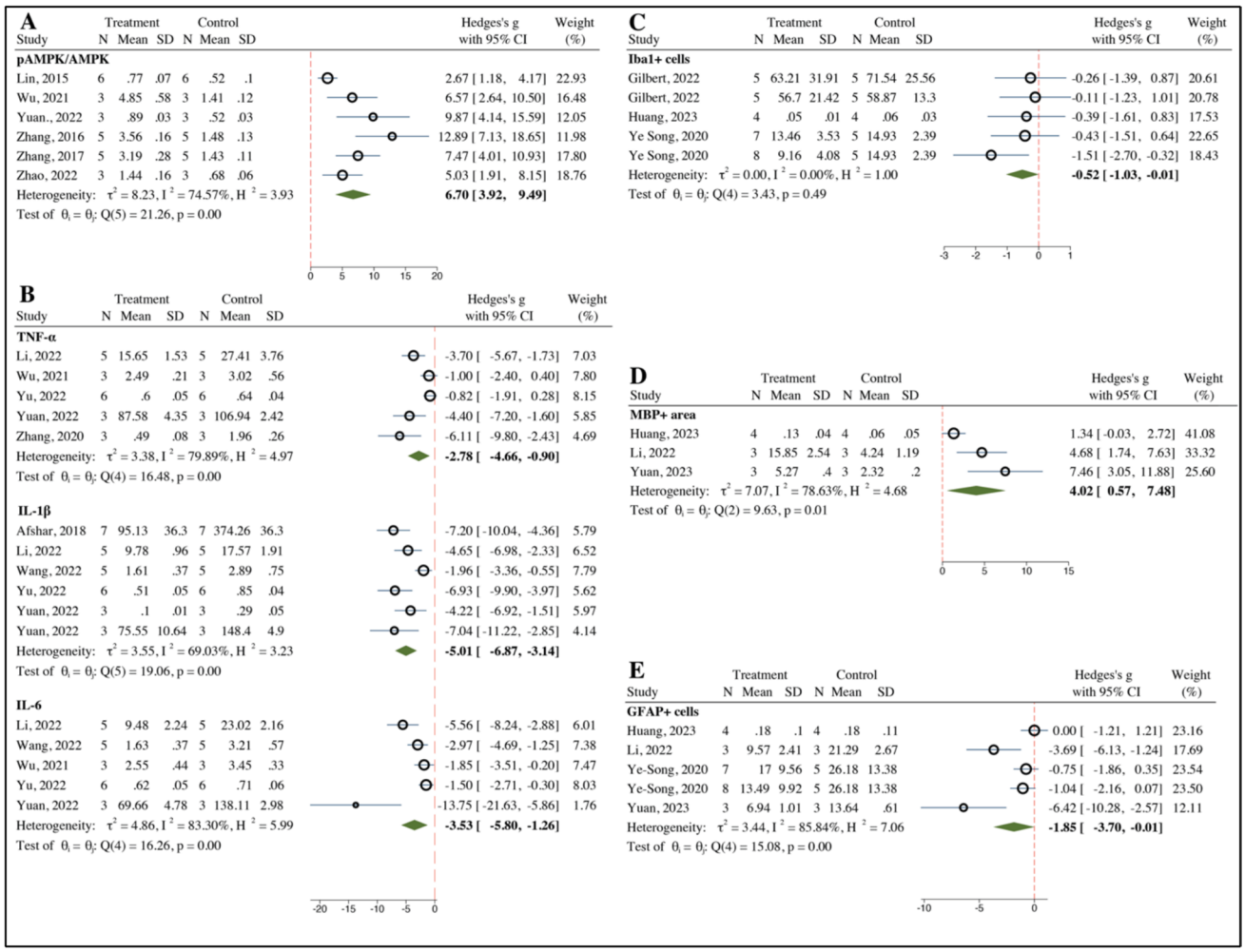

Fig. 4 The forest plot illustrates the efficacy of metformin in (**A**) metabolic, **B** inflammatory, **C** microglial activation, **D** myelination, and (**E**) astrogliosis parameters following spinal cord injury. SD, standard deviation; CI, confidence interval

flux, and reduced apoptosis and lesion size. Consequently, metformin treatment resulted in increased spared tissue and improved neuronal survival.

Metformin is widely recognized as an activator of AMPK signaling, a critical cellular energy sensor, and a metabolic regulator [11, 23]. Our research demonstrated a significant rise in the p-AMPK/AMPK ratio, confirming the activation of the AMPK signaling pathway. This pathway is essential for maintaining energy balance by adjusting metabolic processes in response to fluctuations in cellular energy levels [23].

After neurotraumatic injury, mediators like nitric oxide trigger immune responses, leading to microglial activation and the release of pro-inflammatory factors, including TNF-α, IL-1β, and IL-6. These cytokines cause increased immune cell migration, inflammation, and elevated apoptosis at the injury site [1, 9]. Our meta-analysis demonstrated that metformin significantly reduced the levels of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, which are key mediators in the inflammatory response following SCI. Zhang et al. [67] and Wang et al. [51] suggest that metformin could activate AMPK, which inhibits the inflammatory response by suppressing NF-κB, a key transcriptional regulator of inflammation [51, 67]. Importantly, our study demonstrated a decrease in Iba1 + cells, suggesting metformin's inhibitory role in microglial activation besides cytokine inhibition.

Mitochondrial dysfunction after SCI can increase ROS production, causing lipid peroxidation and cellular damage. SOD and MDA levels reflect the oxidant/antioxidant balance, with SOD binding and breaking down harmful free radicals, while MDA is a byproduct of lipid peroxidation, indicating ROS levels [42, 60]. Our study demonstrated that metformin exhibits antioxidant effects by increasing SOD and reducing MDA levels. Wang et al. [53] and Mohagheghi et al. [32] propose that metformin may suppress ROS production and enhance antioxidant defenses through pathways such as AMPK, Nrf2/ARE, and PI3K/Akt. These pathways

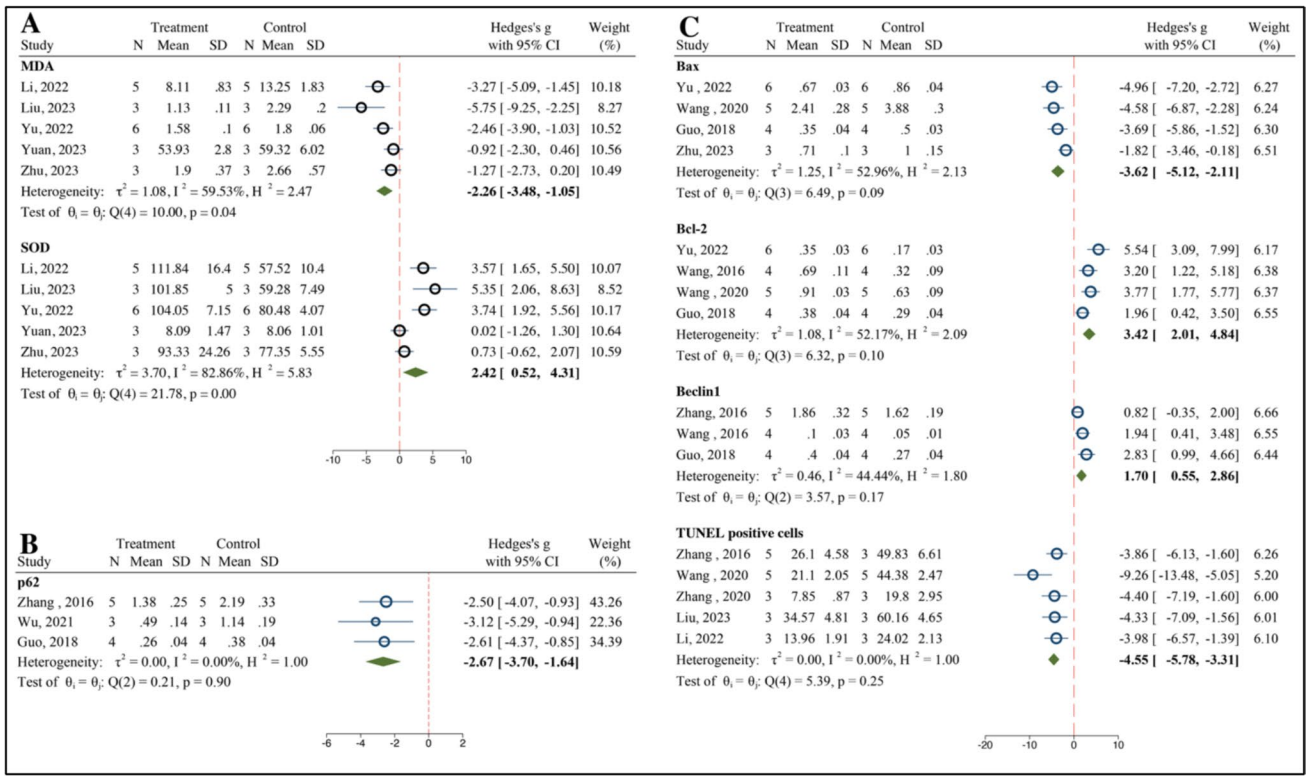

Fig. 5 The forest plot illustrates the efficacy of metformin in (**A**) oxidative stress, **B** autophagic, and (**C**) apoptosis parameters following spinal cord injury. SD, standard deviation; CI, confidence interval

regulate the expression of antioxidant enzymes, including heme oxygenase-1 (HO-1) and NADH dehydrogenase quinone 1 (NQO1)(24,62).

In neurodegenerative diseases, glial scar formation by reactive astrocytes is a major inhibitor of neuroregeneration [1]. Our study demonstrated that metformin therapy reduced GFAP-positive cells, suggesting its potential to modulate astrogliosis. Additionally, SCI disrupts myelin sheaths, exposing axons and blocking nerve conduction [58]. Notably, the metformin group exhibited a significant increase in MBP + area, indicating metformin's ability to promote remyelination after SCI. While the precise mechanisms remain to be fully elucidated, metformin's known effects on cellular metabolism and anti-inflammatory and antioxidant properties may play a role in promoting a myelinating environment.

Autophagy is a cellular process that degrades misfolded proteins, myelin debris, and other components to maintain homeostasis [58, 68]. While autophagic hallmarks are enhanced in acute SCI, it does not necessarily indicate increased autophagy flux, which is defined as the progression from autophagosome formation to cargo degradation in lysosomes. Increased autophagosome or LC3 levels can reflect either autophagy induction or a block in the degradation pathway [56, 68]. Notably, our study showed metformin enhanced LC3 protein abundance while decreasing p62 and ubiquitinated proteins compared to the SCI group, suggesting metformin may restore lysosome function and the autophagy-lysosome pathway. Studies propose that metformin might promote autophagy by phosphorylating AMPK and inhibiting the mTOR signaling pathway [14, 51, 58, 68].

Apoptosis, or programmed cell death, plays a crucial role in the development of secondary injury after SCI [60]. Mitochondria-associated cell death is a critical mechanism triggered by SCI, involving key proteins, such as Bcl-2, Bax, and the executioner Caspase. Cleaved-caspase 3 and Bax are pro-apoptotic factors that induce apoptosis, while Bcl-2 is an anti-apoptotic factor that inhibits apoptosis [12, 60]. Our assessment using Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assays, which detect apoptotic cells undergoing extensive DNA degradation during late-stage apoptosis [19], demonstrated a decrease in the groups treated with metformin, further confirming its inhibitory role in the apoptotic pathway. However, it is important to recognize that metformin's anti-apoptotic effects may not be solely attributable to the direct inhibition of apoptotic pathways but may also result from a combination of interconnected mechanisms (including inflammation, autophagy, and oxidative stress).

Metformin's ability to inhibit inflammation, microglial activation, oxidative stress, astrogliosis, promote myelination

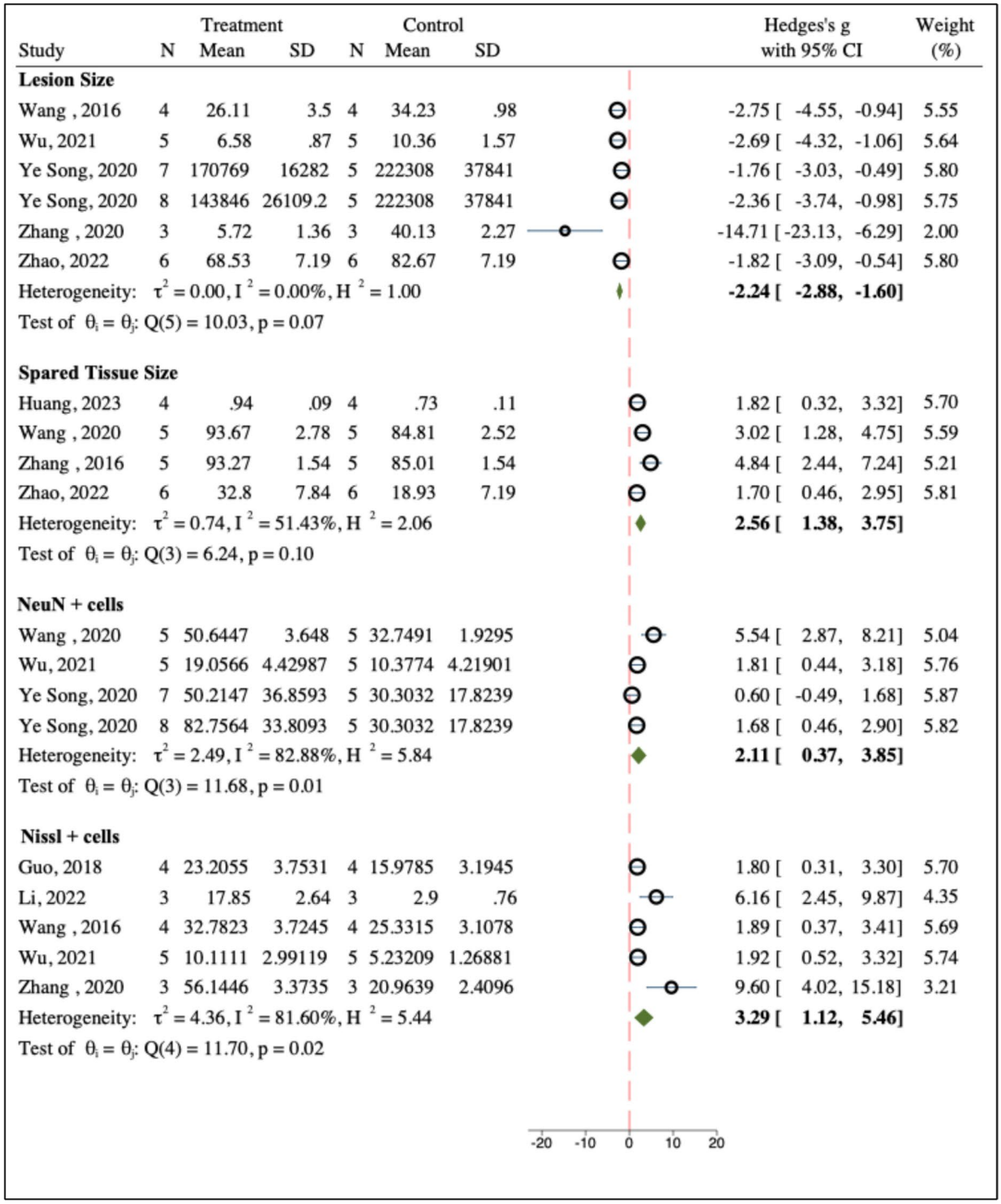

Fig. 6 The forest plot for the efficacy of metformin in lesion characteristics. SD, standard deviation; CI, confidence interval

autophagic flux, and reduce apoptosis and lesion size can explain the observed recovery of locomotion in meta-analyses. Subgroup analyses revealed that metformin administration during the acute phase was more effective, rationalizing that early intervention can prevent microglial/macrophage proliferation, reduce their activation and infiltration, and minimize glial scar formation and lesion area. While a systematic review by Zhou et al. (2021), including 12 studies, demonstrated lower locomotion recovery at metformin doses above 50 mg/kg [62], our study with 23 included studies did not find a significant difference between doses, and the results might be because of low number of studies.

Table 4 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
Afshar, 2018	U	L	U	L	U	L	L	L	L	L
Astaraky, 2022	U	L	U	L	U	U	U	L	L	L
Gilbert, 2022	U	L	U	L	U	U	L	L	L	L
Guo, 2018	L	L	U	L	U	L	L	L	L	L
Huange, 2023	L	L	U	L	U	L	U	L	L	L
Li, 2022	L	L	U	L	U	L	L	L	L	L
Lin, 2015	U	L	U	L	U	L	L	L	L	L
Liu, 2023	U	L	U	L	U	U	U	L	L	L
Wang, 2016	L	L	U	L	U	L	L	L	L	L
Wang, 2018	U	L	U	L	U	L	L	L	L	L
Wang, 2020	U	L	U	L	U	L	L	L	L	L
Wang, 2022	U	L	U	L	U	U	L	L	L	L
Wang, 2023	L	L	U	L	U	L	L	L	L	L
Weng, 2019	U	L	U	L	U	L	L	L	L	L
Wu, 2021	L	L	U	L	U	L	U	L	L	L
Ye-Song, 2020	L	L	U	L	U	L	U	L	L	L
Yu, 2022	L	L	U	L	U	L	U	L	L	L
Yuan, 2022	U	L	U	L	U	L	L	L	L	L
Yuan, 2023	U	L	U	L	U	U	U	L	L	L
Zhang, 2016	L	L	U	L	U	L	L	L	L	L
Zhang, 2017	U	L	U	L	U	L	L	L	L	L
Zhang, 2020	L	L	U	L	U	L	L	L	L	L
Zhao, 2022	L	L	U	L	U	L	L	L	L	L
Zhu, 2023	U	L	U	L	U	L	L	L	L	L

L Low risk; *U* Unclear

Neuropathic pain is a common and debilitating symptom experienced by SCI patients, characterized by persistent hypersensitivity (allodynia) or exaggerated response to nociceptive stimuli (hyperalgesia) [43]. Post-SCI neuronal-glial interactions, maladaptive synaptic plasticity, cellular signaling, and hyperactivity of sensory neurons contribute to heightened neuropathic pain. Additionally, apoptosis might trigger structural changes in neurons, increasing nociceptive system sensitivity and inducing hyperalgesia or allodynia [1, 48]. In our study, metformin administration was associated with a reduction in mechanical and thermal hypersensitivity in SCI rodents. This analgesic effect may be linked to metformin's modulation of neuroinflammatory pathways, including the inhibition of astrogliosis and apoptosis. However, it is important to acknowledge that the precise mechanisms underlying metformin's antinociceptive properties are multifaceted. Studies have indicated that metformin may alleviate neuropathic pain by activating AMPK, which in turn inhibits the activation of astrocytes and microglia, leading to reduced neuroinflammation. Additionally, metformin's influence on synaptic transmission and plasticity may contribute to its analgesic effects [27, 47]. Further research is necessary to delineate the specific pathways through which metformin exerts its effects on neuropathic pain following SCI.

Rodent models serve as essential pre-clinical platforms for studying SCI due to their anatomical and functional similarities with human motor pathways, particularly in the corticospinal tract, where they exhibit comparable mechanisms of axonal regeneration, neuroplasticity, and therapeutic responses [18, 34, 40]. The promising neuroprotective effects of metformin observed in these rodent models have significant implications for human SCI treatment, providing a strong rationale for clinical trials to evaluate its efficacy in improving neurological outcomes. Given metformin's established safety profile and widespread clinical use for type 2 diabetes, repurposing it for SCI treatment could expedite the availability of a novel therapeutic option. Future clinical studies should focus on optimal dosing strategies, treatment initiation windows, and long-term effects of SCI patients.

Limitations

It is important to note that our study has several limitations. First, significant heterogeneity was present, which we addressed through detailed sub-group analyses. Second, measures such as BBB, BMS, withdrawal latency, and threshold are subjective and susceptible to rater bias. Another limitation of this study is the specificity of markers used to assess certain cells or pathways. These markers may also be involved in other pathways or cell types, which could affect the interpretation of results. Furthermore, due to the limited number of studies focusing on specific types of injuries, such as cervical spinal cord injury, we were unable to perform a subgroup analysis for these conditions. Besides, our meta-analysis couldn't pinpoint exactly which part of each pathway metformin acts on because of the limited number of studies. Finally, the present study only includes studies conducted on normal animals without risk factors for cardiovascular diseases, such as aging, diabetes, obesity, hyperlipidemia, and hypertension. Consequently, our results cannot be generalized to animals with these risk factors. More animal studies and human RCTs are required to address these issues and confirm the therapeutic effect of metformin conditioning on SCI.

Conclusion

This meta-analysis supports the therapeutic potential of metformin in SCI rodent models, demonstrating its role in promoting locomotion recovery and pain relief, as well as modulating multiple pathways implicated in secondary injury cascades. Specifically, metformin activated the AMPK signaling pathway, exerting anti-inflammatory and antioxidant effects, inhibited microglial activation and astrogliosis, promoted myelination and autophagy flux, and reduced apoptosis and lesion size. Consequently, metformin treatment resulted in increased spared tissue and improved neuronal survival. These findings support the translational potential of metformin for clinical SCI management.

Author contributions Conceptualization: MY, VRM; Data curation: MV, MY, FT, AZ, HZ; Formal analysis: AA, HZ, MY; Funding acquisition: MY; Methodology: AA, MY, HZ, VRM; Project administration: MY, VRM; Visualization: AA; Writing—original draft: AA, HZ, MV; All authors reviewed the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval NA.

Competing interests The authors declare no competing interests.

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