Review Article

Ginseng extract and ginsenosides improve neurological function and promote antioxidant effects in rats with spinal cord injury: A meta-analysis and systematic review

Kim Sia Snga, b, Gan Li a, b, Long-yun Zhou c, Yong-jia Song a, b, Xu-qing Chen d, Yong-jun Wang a, b, c, Min Yao a, b, **, Xue-jun Cui a, b, *

* Institute of Spine Disease, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China
** Key Laboratory of Theory and Therapy of Muscles and Bones, Ministry of Education (Shanghai University of Traditional Chinese Medicine), Shanghai, China
** Rehabilitation Medicine Center, Jiangsu Provincial People’s Hospital, Jiangsu, China
** Department of Otolaryngology, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, China

A B S T R A C T

Spinal cord injury (SCI) is defined as damage to the spinal cord that temporarily or permanently changes its function. There is no definitive treatment established for neurological complete injury patients. This study investigated the effect of ginseng extract and ginsenosides on neurological recovery and antioxidant efficacies in rat models following SCI and explore the appropriate dosage. Searches were done on PubMed, Embase, and Chinese databases, and animal studies matched the inclusion criteria were selected. Pair-wise meta-analysis and subgroup analysis were performed. Ten studies were included, and the overall methodological qualities were low quality. The result showed ginseng extract and ginsenosides significantly improve neurological function, through the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale (pooled MD = 4.40; 95% CI = 3.92 to 4.88; p < 0.00001), significantly decrease malondialdehyde (MDA) (n = 290; pooled MD = −2.19; 95% CI = −3.16 to −1.22; p < 0.0001) and increase superoxide dismutase (SOD) levels (n = 290; pooled MD = 2.14; 95% CI = 1.45 to 2.83; p < 0.00001). Both low (<25 mg/kg) and high dosage (>25 mg/kg) showed significant improvement in the motor function recovery in SCI rats. Collectively, this review suggests ginseng extract and ginsenosides has a protective effect on SCI, with good safety and a clear mechanism of action and may be suitable for future clinical trials and applications.

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1. Introduction

Spinal cord injury (SCI) is a disastrous condition that is defined as damage to the spinal cord that temporarily or permanently changes its function [1]. SCI can be caused by a traumatic event leading to immediate mechanical disruption and dislocation of the vertebral column, subsequently causing compression or transection of the spinal cord. Such injury damages the neurons and oligodendrocytes, disrupts the vasculature, and compromises the blood-spinal cord barrier [1]. These events eventually result in further spinal cord damage and severe neurological dysfunction due to a sustained cascade of secondary injuries, including cell dysfunction, cell death, apoptosis, and demyelination accompanied by severe hemorrhage due to blood vessel injuries, destruction of the microvascular supply of the spinal cord, neurotoxicity, or glial scarring. These conditions encourage cell permeabilization and activate pro-apoptotic signaling [2–4]. Subsequently, an influx of inflammatory cells (macrophages, neutrophils, and lymphocytes), cytokines (tumor necrosis factor (TNF) and interleukin (IL)-1β), and vasoactive peptides flood the spinal cord, contributing to the ischemic injury and neuroinflammation [5].

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Patients with SCI may experience partial or complete loss of sensorimotor function, paralysis, or neurogenic bowel or bladder dysfunction; others experience fatal symptoms such as compromised respiratory function, disruption of the sympathetic nervous system, hypotension, and bradycardia [1]. The incidence of SCI varies worldwide. The estimated incidence of traumatic SCI (TSCI) increased from 133 to 226 thousand globally in 2007 to 3.6 to 195.4 cases per million in 2014 [6,7]. The incidence has decreased or has remained stable over time in developed countries due to the implementation of preventive measures based on the epidemiology of TSCI [8]. Previous studies in Japan and Spain reported that SCI affects younger patients due to road traffic accidents, but recent studies report that TSCI now affects mainly older adults due to falls [9,10].

Various meta-analyses have evaluated the potential efficacies of SCI treatments such as natural products [11,12], medical drugs [13], and transplantation of stem cells [14]. However, there is currently no established definitive treatment for patients with complete neurological injury in the clinical setting [15,16]. Currently available treatment methods include the maintenance of adequate spinal cord perfusion, intravenous administration of a large dosage of methylprednisolone sodium succinate, and decompressive surgery. Neuron-rehabilitation treatments such as body-weight supported locomotor training and physical therapy generally achieve only limited improvements in patients with SCI [17].

Ginsenosides are a group of natural steroid glycosides and triterpene saponins. Approximately 40 ginsenoside compounds have been identified [18], with the ginsenosides Rb1, Rg1, Rg3, Re, Rd, and Rh1 being commonly studied [19–21]. These compounds are found almost exclusively in the plant genus Panax, but are mainly derived from Panax ginseng roots and processed via purification of the column or high-performance liquid chromatography [22]. Ginsenosides possess the bioactivities of antioxidation [23], neuroprotection [24,25], promotion of neurite outgrowth [26], anti-inflammation [27], antitumor [28], and memory improvement [29]. In addition, various ginsenoside compounds improve the condition of patients with stroke and neuronal damage due to oxidative stress and neurotoxicity. Ginsenoside Rb1 is beneficial in the treatment of cerebral ischemia and protects the blood–brain barrier under ischemic stroke condition [30,31]. Ginsenoside Rg1 prevents apoptosis of astrocytes by reducing brain edema and infract volume in patients with middle cerebral artery occlusion [32], and protects the hippocampus from neuronal damage by reducing NADPH oxidase 2–mediated reactive oxygen species (ROS) production [33]. Various studies have shown that ginsenosides Rb2, Rg2, Rg3, and Rd prevent neurotoxicity by reducing the level of neurotoxins such as trimethyltin or glutamate [34–36]. Ginsenosides also have a regulatory effect on the phosphatidylinositol 3-kinase (PI3K)/AKT and MAPK/ERK signaling pathways [37]. Furthermore, ginsenoside Rd promotes neural regeneration and axonal outgrowth by upregulating GAP-43 and increasing the expression of vascular endothelial growth factor and brain-derived neurotrophic factor in hypoxic PC 12 cells [26,38]. Therefore, with the vast array of ameliorative effects such as antioxidation, neuroprotection, promotion of neurite outgrowth, and anti-inflammation, Panax ginseng and its major component, ginsenosides, could potentially reduce secondary complications in patients with SCI.

A systematic review aims to answer a particular research question by collecting empirical evidence that meets the eligibility criteria, while a meta-analysis is a subset of a systematic review. A meta-analysis systematically assesses the results of previous research by quantitatively deriving conclusions and assesses the strength of evidence regarding a specific disease and treatment [39]. The results of a meta-analysis can enhance the accuracy of estimates of effect, resolve controversies from current conflicting studies, and generate new hypotheses [39].

The present study aimed to investigate the effect of ginseng extract and ginsenosides on neurological recovery and their antioxidant efficacies in a rat model of SCI by assessing the results of previous related research, and to define the effectiveness of ginseng extract and ginsenosides in the treatment of SCI.

2. Methods

2.1. Study selection

The following electronic databases were searched from inception to April 1, 2019 to identify relevant animal studies without language restrictions: PubMed, Embase, China National Knowledge Infrastructure, SinoMed, VIP, and WanFang. The reference lists of the included studies were screened to identify any additional relevant studies. MeSH terms such as “spinal cord injury” “spinal cord diseases” “spinal cord contusion” “spinal cord laceration” “spinal cord transection” “spinal cord trauma” “ginsenosides” “ginsenoside Rb1” “ginsenoside Rg1” and “ginseng saponin” were used during the search process. Appendix 1 contains the PubMed database search strategy.

Two reviewers (K.S.S. and M.Y.) independently screened the abstracts and full texts of the retrieved studies. Any disagreements were resolved through discussion with a third reviewer (X.J.C.).

2.2. Eligibility criteria

2.2.1. Types of studies

All studies assessing the effect of ginseng extract and ginsenosides in rats with SCI were included. Clinical case reports or only in vitro studies were excluded.

2.2.2. Types of participants

There were no restrictions regarding the age, sex, or strain of laboratory rats. Rats that underwent contusion or compression to induce SCI were included. Rat models of SCI created using laceration, transection, nontraumatic ischemia-reperfusion, photochemical reaction, traumatic root avulsion, dorsal root entry zone damage, and genetic modification were excluded.

2.2.3. Types of intervention

Studies evaluating ginsenosides of any type compared with placebo controls were included. There were no restrictions on the dosage, formulation, administration route, and timing of ginsenosides. Placebo controls were physiological saline or no treatment.

2.3. Type of outcome measures

2.3.1. Functional evaluation

The rats’ motor function was evaluated with the Basso, Bresnahan (BBB) locomotor rating scale, inclined plane test, and open field test. The BBB locomotor rating scale assessment is widely used to evaluate the post-injury motor behavior of animals [40], and is validated and widely accepted [41–43]. Assessors observe the hindlimb movement of animals and assign a BBB scale score ranging from 0 (complete paralysis) to 21 (normal locomotion). The inclined plane test evaluates the rats’ ability to maintain their position for 5 seconds at certain degrees of inclination to assess the motor behavior recovery after SCI [44]. The open field test is performed to evaluate locomotor deterioration in animal models of neuromuscular disorders [45]. The rats are placed in an open arena surrounded by walls to measure the open field activity, such as the total distance traveled through a grid of infrared light [46–48].
2.3.2. Biochemical analysis
During SCI, oxidative injury in the spinal cord produces lipid peroxidation (LPO), which contributes to cell damage. LPO is measured based on the levels of malondialdehyde (MDA) and superoxide dismutase (SOD). As ginsenosides stimulate the production of SOD and reduce the activity of MDA, both indicators are used to assess the efficacy of ginsenosides in animal models of SCI [49,50]. MDA is the most studied byproduct of the process of LPO of polyunsaturated fatty acids and is often used as a marker of LPO [51]. SOD is an enzyme produced during the inflammatory response that limits further damage caused by ROS elements [52]; increasing evidence has shown that pharmacological products [49] and antioxidant treatments [53] reduce the impact of secondary injury by limiting ROS. The MDA and SOD levels at 7 ± 3 days after SCI were analyzed in the present study.

2.3.3. Data extraction
The details of the included studies were independently extracted by 2 authors (K.S.S. and M.Y.). Extracted data included the name of the first author, publication year, animal strain, weight, and sex, number of animals in each group, method used to induce SCI, SCI levels, ginsenoside administration (including dosage, method, and timing), and measured outcomes. The mean and standard deviation of each variable were extracted for comparisons. In accordance with the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions, for studies with multiple intervention groups, the experimental group was combined to enable a single pair-wise comparison. The present review compared the effect of different dosages on the recovery from SCI, with 25 mg/kg and low (<25 mg/kg) dosages of ginsenosides. Intervention groups in the respective dosage groups were combined. If a study had 2 different dosage groups, the shared control group was divided approximately evenly among the comparisons. GetData Graph Digitizer 2.24 was used to interpret graph data. Disagreements were resolved via discussion with a third reviewer (X.J.C.).

2.3.4. Risk of bias assessment
The reporting quality and design of studies were assessed using the initial Stroke Therapy Academic Industry Roundtable (STAIR) guidelines [54]. In 2009, a 7-point checklist was released in accordance with the Recommendations for Ensuring Good Scientific Inquiry [55].

2.3.5. Statistical analysis
Data from all included studies were summarized, and data were analyzed using RevMan 5.3 software. Studies involving a direct comparison of the ginsenoside group versus the control group were analyzed using the pair-wise meta-analysis method. Mean differences (MD) were used for outcomes using the same unit, while standardized MD was used for outcomes using different units. Cochrane’s I² value was used to identify heterogeneity between the groups. Heterogeneity was presumed in the event that the I² value was more than 50% [56]. Fixed-effect models were used for studies with homogenous clinical and statistical analyses, while random-effect models were used for studies with heterogeneous clinical and statistical analyses [56]. A subgroup meta-analysis was done of high ($≥25$ mg/kg) and low ($<25$ mg/kg) ginsenoside dosages. A pair-wise meta-analysis with subgroup analysis was done to evaluate the relative effects of each intervention with other effects. The effect of interventions was determined by obtaining the MD of postintervention values through a comparison of the ginsenoside and control groups [56]. A line graph was constructed by GraphPad Prism 8.0.2 software to highlight the BBB score improvements in both groups. The axes were the BBB score (points) against the time after SCI (days), with available data on day 1, 2, 3, 4, 7, 10, 14, 21, and 28 after SCI.

3. Results

3.1. Description of included studies
The study selection process is shown in Fig. 1. The search strategy identified 255 studies. Duplicate studies were eliminated, and further screening resulted in 18 studies being selected for full-text screening. Ten of these 18 studies were included; the 8 excluded studies were 1 in vitro experiment [57], 3 studies that reported intervention combining ginsenosides with other treatments [58–60], and 4 studies that reported intervention including the whole ginseng root [61–64]. Among the 10 included studies, 6 were published in Chinese, while 4 were in English [65–74].

3.2. Characteristics of included studies
The characteristics of the included studies are summarized in Table 1. Of the 10 studies that met the inclusion criteria, 9 studies used Sprague-Dawley rats, while 1 study used Wistar rats. The sample size ranged from 24 to 108. A TSCI model was used in all included studies; 8 studies used a weight-drop impactor, and 2 studies used the aneurysm clip compression method. All studies established SCI from T7 to T12.

Among the studies, 8 reported both functional and biochemical outcomes, while 2 reported only biochemical outcomes. The BBB scale was used in 8 studies, while the inclined plane test, open field locomotor score, brain temperature, and water maze test were each used in 1 study. The duration of evaluation ranged from 24 hours to 28 days.

3.3. Risk of bias assessment
The risk of bias assessment for all included studies is shown in Table 2. Generally, the STAIR assessment reflected a relatively low methodological quality of the studies. None of the included studies reported the sample size calculation, potential conflicts of interest, and study funding. Five studies described the inclusion and exclusion criteria for the SCI model. Nine studies reported randomization, while no studies reported allocation concealment. The exclusion of animals from the analysis was reported in 2 studies. Blinded assessment of outcomes was reported in 4 studies.

3.4. Ginsenoside efficacy in neurological recovery

3.4.1. Change in the BBB score after SCI
For the analysis of locomotor recovery, 8 studies reported the BBB score after treatment. The ginsenosides group showed better improvement in the BBB score than the control group, reaching a peak on the 21st day after SCI (BBB = 14.81 ± 1.1) (Fig. 2). The BBB scores in both the ginsenoside and control groups increased rapidly on day 1 and 2 after SCI. The increasing trend slowed down from the 3rd day (pooled MD = 1.72; 95% CI = 1.53 to 1.90; p < 0.00001) to the 10th day after SCI (pooled MD = 2.36; 95% CI = 1.70 to 3.02; p < 0.00001). The BBB score on the 28th day after SCI was significantly better in the ginsenoside group than the control group (pooled MD = 4.40; 95% CI = 3.92 to 4.88; p < 0.00001).

3.4.2. Subgroup analyses of the effect of ginsenosides
Subgroup analyses were performed based on the dose of ginsenosides and the method used to establish the SCI model. Day 14 after SCI was selected as the timepoint for analysis. Analyses of rat
strains, injury location, and administration methods could not be carried out because these variables were consistent in the included studies.

3.4.3. Effect of the ginsenoside dosage on the BBB score
The subgroup analysis included 7 studies. The administration of ginsenosides at a high dosage of ≥25 mg/kg (pooled MD = 4.93; 95% CI = 3.16 to 6.71; p < 0.00001) resulted in significantly better motor function recovery in rats with SCI compared with ginsenosides at a low dosage of <25 mg/kg (pooled MD = 3.01; 95% CI = 2.28 to 3.75; p < 0.00001) (p = 0.05) (Fig. 3).

3.4.4. Effect of the ginsenoside dosage on the inclined plane test
Only 1 included study used the inclined plane test to evaluate the neurological recovery of rats with SCI after ginsenoside administration [72]. The administration of ginsenosides at a dosage of 5 mg/kg after SCI resulted in a significant improvement in motor function recovery in the intervention group compared with the control group (p < 0.01).

3.4.5. Effect of the ginsenoside dosage on the open field test
Only 1 included study used the open field test to evaluate the neurological recovery of rats with SCI after ginsenoside administration [73]. The administration of ginsenosides at a dosage of 6.0 µg/day resulted in significantly improved motor function recovery after SCI in the intervention group compared with the control group (p < 0.01).

3.4.6. Effect of the SCI establishment method on the BBB score
The contusion method of establishing SCI (pooled MD = 3.46; 95% CI = 2.70 to 4.21; p < 0.00001) and compression method of establishing SCI (pooled MD 3.34; 95% CI = 2.62 to 4.06; p < 0.00001) did not affect the rate of improvement of motor function recovery (p = 0.88) (Fig. 4).

3.4.7. Antioxidant effects of ginsenosides
Seven studies (n = 290) evaluated the MDA and SOD levels; the levels were reported on day 7 in 2 studies, day 14 in 4 studies, and day 28 in 1 study. The random-effect model analysis showed that the ginsenoside group had a significant reduction in the MDA level (pooled MD = –2.19; 95% CI = –3.16 to –1.22; p < 0.0001) (Fig. 5) and a significant increase in the SOD level (pooled MD = 2.14; 95% CI = 1.45 to 2.83; p < 0.00001) (Fig. 6) compared with the control group.

4. Discussion

4.1. Summary of evidence
To the best of our knowledge, no prior meta-analysis has quantitatively evaluated the efficacy of ginsenosides in treating SCI. The present meta-analysis demonstrated that compared with the control group, the group treated with ginsenosides achieved significant improvements in motor function that were directly correlated with time, with the most significant difference in improvement seen on day 21 after SCI. Although the motor function recovery remained stable from 21 days after SCI, the significant improvement in the ginsenoside group versus the control group shows that ginsenosides are effective in treating SCI. Both high and low dosages of ginsenosides improved the motor function compared with the control group. However, better improvement was seen after the administration of a high dosage of ginsenosides (≥25 mg/kg) compared with a low dosage (<25 mg/kg). The results of biochemical analysis, such as the MDA and SOD levels, showed that the antioxidant effect was significantly greater in the ginsenoside group than the control group.

4.2. Possible mechanism of ginsenosides in SCI
Ginseng is the root and rhizome of Ginseng Radix et Rhizoma, also known as Panax ginseng Meyer in the genus Panax of the Araliaceae family [75]. After the oral administration of ginseng, the gut microbiota converts ginsenoside into its metabolites. Generally, the metabolism of ginsenosides occurs in the gastrointestinal tract after oral administration [76]. Ginsenosides were first isolated in the 1960s and have been extensively studied because ginseng is widely used worldwide. Ginsenosides have a similar basic structure; almost all ginsenosides have 30 carbon atoms arranged in four rings of steroid nuclei [77]. Ginsenosides are divided into 4 types based on the amount and position of the sugar moiety: panaxadiol (Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2), panaxatriol (Re, Rg1, Rg2, Rh1), oleanolic acid (Ro), and ocottillol (Rs) [78,79].
The ginsenosides Rb1, Rg1, Rg3, Re, Rd, and Rh1 are commonly studied [19–21], and all studies included in this review evaluated at least one of these ginsenosides. Clinical research has shown that ginsenosides have distinct neuroprotective (Rb1, Rg1, Rg2, Rh1) [86,87] and antiosteoporotic benefits (Rb2) [92].

Under normal circumstances, the human body produces byproducts such as ROS through numerous physiological and biochemical processes [94]. However, TSCI induces acute inflammatory responses triggered by the innate immune system, including systemic vasodilation, vascular leakage, and leukocyte emigration [95]. Secondary spinal cord injury then occurs due to the infiltration of leukocytes and glial cell activation [96]. The presence of polymorphonuclear neutrophils enhances the generation of ROS during inflammation and contributes to spinal cord swelling, which leads to further compression and worsens the injury [97]. The glial cell activation characterized by neuroinflammation is fueled by the vast infiltration of inflammatory cells and cytokines such as TNF-α, IL-1β, and IL-6 in the spinal cord [98]. Furthermore, as the spinal cord contains a large amount of unsaturated fatty acids, it has active cell metabolism and a low antioxidant capacity [99]. Therefore, SCI causes a severe oxidative stress metabolism and contributes to spinal cord injury [100].

MDA is a byproduct of the LPO process and is indicative of the level of LPO and cell damage by ROS. The mechanism by which ginsenosides relieve secondary spinal damage may be inhibition of the infiltration of calcium ions from the extracellular matrix into the damaged cells [101,102]. MDA staining, hematoxylin-eosin staining; SOD, Superoxide Dismutase; MDA, malondialdehyde.
convert hydrogen peroxide into water and prevent it from contributing to LPO \[103\]. Therefore, MDA and SOD are currently recognized as reliable indicators of oxygen free radical levels after SCI \[104\]. Hence, during the event of SCI, if the activity of SOD could be increased and the production of MDA could be reduced, this could potentially help to reduce secondary damage to the spinal cord.

The included studies showed that the ginsenosides Rg1 \[65,69,70\], Rb1 \[66,71\], Rd \[68\], and ginseng saponins \[74\] effectively reduced the production of MDA and increased the activity of SOD in rats with SCI. Furthermore, the ginsenosides Rg1 and Rb1, and ginseng saponin upregulated the Bcl-2/Bax ratio and downregulated the caspase-3 expression. The ginsenosides Rg1 and Rd also reduced the expression of TNF-\(\alpha\), IL-1\(\beta\), IL-6, and IL-10. These findings are similar to the findings of a study investigating the effect of various ginsenosides in protecting against cerebral ischemic reperfusion injury \[105\]. The ginsenosides Rg1 \[32\], Rb1 \[106\], and Re \[107\] reduced MDA production and increased SOD activity, while the ginsenosides Rd \[38\] and Rg3 \[108\] upregulated the

### Table 2

| Study       | Sample-size calculation | Inclusion and exclusion criteria | Randomization allocation concealment | Reporting of animals excluded from analysis | Blinded assessment of outcome | Reporting potential conflicts of interest and study funding |
|-------------|-------------------------|----------------------------------|--------------------------------------|--------------------------------------------|-----------------------------|--------------------------------------------------------|
| Sun, J.Z.   | Unclear                 | Unclear                          | Low                                  | Unclear                                   | Unclear                     | Unclear                                               |
| Liu, X.     | Unclear                 | Unclear                          | Low                                  | Unclear                                   | Unclear                     | Unclear                                               |
| Wang, P.    | Unclear                 | Unclear                          | Low                                  | Unclear                                   | Low                         | Unclear                                               |
| Cong, L.    | Unclear                 | Low                              | Low                                  | Unclear                                   | Low                         | Unclear                                               |
| Sun, J.Z.   | Unclear                 | Unclear                          | Low                                  | Unclear                                   | Low                         | Unclear                                               |
| Liu, Y.L.   | Unclear                 | Low                              | Low                                  | Unclear                                   | Low                         | Unclear                                               |
| Li, Q.      | Unclear                 | Low                              | Low                                  | Unclear                                   | Unclear                     | Unclear                                               |
| Song, Y.X.  | Unclear                 | Low                              | Unclear                             | Unclear                                   | Low                         | Unclear                                               |
| Sakanaka, M.| Unclear                 | Low                              | Low                                  | Unclear                                   | Unclear                     | Unclear                                               |
| Guo, D.Q.   | Unclear                 | Low                              | Low                                  | Unclear                                   | Low                         | Unclear                                               |

SD, Sprague-Dawley; T, thoracic vertebrae; SCI, spinal cord injury; GS Rd, Ginsenoside Rd; i.p., intraperitoneal; BBBS, Basso-Beattie-Bresnahan locomotor rating scale; HE staining, hematoxylin-eosin staining; SOD, Superoxide Dismutase; MDA, malondialdehyde.

Fig. 2. The Basso, Bettie, Bresnahan (BBB) score of the ginsenoside and control groups at different time points after spinal cord injury (SCI).

Fig. 3. Effect of different dosages of intraperitoneally injected ginsenosides on the Basso, Bettie, Bresnahan (BBB) score.
P13K/AKT and ERK1/2 pathways, and reduced caspase-3 expression.

Studies have also shown that both panaxadiol and panaxatriol ginsenosides inhibit the expression of TLR4 and MyD88, and activate SIRT1 to down regulate the expression of NF-κB [109]. This mechanism reduces the release of inflammatory factors such as TNF-α, IL-1β, and IL-6. Panaxadiol ginsenoside (Rb1 and Rg3) have a stronger effect than panaxatriol ginsenosides (Rg1 and Re) and reduce both cerebral ischemic injury and neurological deficits in ischemic rats [109]. Other studies have produced similar results, showing that both panaxadiol ginsenosides (Rg1, Rg2) and panaxatriol ginsenosides (Rg1, Re) reduce the release of inflammatory factors, while panaxatriol also reportedly regulates microglia and astrocyte activation [113,114]. In addition, studies have shown that both panaxadiol and panaxatriol ginsenosides inhibit inflammasome activation by suppressing the activation of NLRP3 and AIM2 in inflammasomes during a macrophage-mediated inflammatory response in a dose-dependent manner [115]. In the regulation of oxidative stress, panaxatriol ginsenosides increase the amount of glutathione and reduce the production of MDA in cardiomyocytes with ischemic reperfusion [116]. Moreover, panaxadiol ginsenosides are superior to panaxatriol ginsenosides in lowering apoptosis rates [109,117,118], while both panaxadiol and panaxatriol ginsenosides are cardioprotective and protect against cerebral ischemic injury. However, both panaxadiol and panaxatriol ginsenosides show a diminished cardioprotective trend as the molecular weight and complexity of the compound increases [119]. This is evident by the fact that Rg3 has less cardioprotective capabilities than other panaxadiol ginsenosides such as Rh2, even though the ginsenosides share a similar chemical structure [120,121]. The ginsenosides Rh2 and Rg5 also provide a better protective effect than the ginsenosides Rb1, Rg2, and Rg3 in a cerebral ischemic injury model [109]. The cardioprotective properties tend to diminish in both groups with the increasing molecular weight and complexity of the compound. It is believed that a larger molecular weight inhibits the ability of the compound to cross the cell membrane to act on the mitochondrial permeability transition pore [119]. Furthermore, compounds with high polarity are poorly absorbed [122]. Hence, compounds with a large number of hydroxide groups (such as ginsenosides Rg2 and Rg3) are not cardioprotective [119].
There are currently several clinical trials related to SCI treatment, which can be categorized into physiological approaches, pharmacology, and stem cell treatment [123]. However, there are currently no clinical trials involving the administration of ginsenosides to patients with SCI, and clinical trials involving ginsenosides are scarce [124]. Clinical trials have evaluated ginsenoside intervention in patients with carcinoma in the liver, lung, and gastric system, acute ischemic stroke, rheumatoid arthritis, metabolic syndrome, cardiovascular disease, and blood pressure disorders [124]. Ginsenosides are mostly administered in oral capsule or tablet form, while some trials have evaluated ginsenosides administered through the intravenous infusion route to patients with acute ischemic stroke [125]. Trials have been performed to assess the effect of traditional Chinese and Korean herbal medicines on patients with cervical spondylotic myelopathy who present with symptoms of SCI. However, most such trials have involved a combination of non-surgical interventions such as herbal medicine, tuina or chuna (also known as massage therapy), electro-acupuncture, acupotomy, cervical traction, exercise, and physiotherapy [126–132], while only one trial reported the use of herbal medicine alone [133].

Consistency in the chemical composition and pharmacological properties is essential in any trial evaluating the safety and effective administration of herbal drugs. However, ginseng and other herbal products generally fail to meet this standard [134]. This is most likely due to the poor extraction methods and lack of standardization regarding ginseng preparation [134]. Although chromatographic techniques and marker compounds are used to standardize...
the extraction of herbal products, this method does not guarantee consistent and stable pharmacological activity. Moreover, the production and quality of ginsenoside extracts is largely dependent on the extraction method, as ginsenosides become unstable at high temperatures [135]. Conventional extraction methods often cause thermal destruction of biologically active compounds due to long extraction times and large solvent volumes [136]. However, the supercritical extraction method utilizing CO₂ extracts a richer extract with an environmentally friendly technique due to easy removal of the solvent [137].

4.3. Safety of the consumption of ginseng and ginsenosides

Ginseng, which contains an abundant amount of ginsenosides, generally has a good safety profile [138]. However, there have been reports of adverse events after ginseng administration in randomized controlled trials, such as gastrointestinal discomfort [139,140], insomnia, headache, and chest discomfort [141–143]. Recently, the safety of ginsenosides has been investigated in preclinical studies. Many such studies have evaluated compound K (CK), which is a metabolite of the ginsenosides Rb1, Rb2, and Rc and is not present in ginseng itself [144]. One study showed that although no rats were killed during 26-week repeated-dosage toxicity testing of CK at dosages of 13 mg/kg, 40 mg/kg, and 120 mg/kg, rats in the 120 mg/kg group showed fur loss, weakness, underactive locomotor activity, and increased ALT and ALP levels, suggesting potential hepatotoxicity [145]. However, these adverse effects may be affected by the different sensibility and tolerance of test substances between different species [145]. Another study that performed 26-week repeated-dosage toxicity testing of ginsenoside Rg3 at dosages of 20 mg/kg, 60 mg/kg, and 180 mg/kg reported no rat deaths and no significant toxicological effects [146]. Furthermore, a clinical study of healthy Chinese volunteers showed that a single dose of CK (25–800 mg) or multiple doses of CK (100–400 mg) were well tolerated, without any significant adverse events [147].

4.4. Comparison of different ginsenoside doses

The oral administration of ginsenosides leads to differences in the administered dosage due to the poor absorption of orally administered ginsenosides [148]. Furthermore, the low oral bioavailability of ginsenosides leads to a low plasma concentration [149]. Therefore, all included studies chose to administer ginsenosides intraperitoneally rather than orally.

The included studies with groups that received various dosages of ginsenosides showed that groups that received higher dosages had higher SOD and lower MDA levels than the groups that received lower dosages. Both high and low dosages of ginsenosides resulted in significant improvements in the recovery of motor function after SCI in rats, with a higher dosage resulting in significantly better improvement than a lower dosage of ginsenosides. The ginsenoside Rb1 decreases ROS production [150] and recovers the disproportion of cellular redox enzymes [151]. Furthermore, oxidative stress is regulated by ginsenosides through the PI3K/AKT and Nrf2/HO-1 pathways [152].

4.5. Strengths and limitations of this review

To the best of our knowledge, no previous study has quantitatively analyzed the efficacies of ginseng extract and ginsenosides in SCI. The present meta-analysis included relevant studies retrieved from Chinese databases and analyzed the effect of different dosages of ginsenosides and the effect of different SCI establishment methods on the motor function recovery of rats with SCI.

The present review has several limitations. Although 10 studies were included, 6 were published in Chinese and 4 were in English. As ginseng is one of the most important and commonly used herbs in Asia, including China and Korea [153], these two countries would naturally contribute the most publications related to ginseng worldwide [154]. Therefore, it is inevitable that more than half of the studies included in this review were published in Chinese, which may make it difficult for non-Chinese readers to access the articles. Moreover, the STAIR assessment showed that the overall quality of the included studies was not high. None of the included studies achieved more than 3 out of the 7-point checklist. First, no studies mentioned the calculation of sample size. Some experiments had 36 rats per group, while other had as little as 6 rats per group. For ethical and financial reasons, it is crucial to design animal experiments well to ensure that the obtained data is reliable, especially regarding the sample size. The number of animals per group may be decided by referring to a table specifying the effect size and the respective number of samples [155]. Second, a moderate amount of heterogeneity was observed in the included studies. The heterogeneity among the included studies only involved different dosages of ginsenosides and different SCI establishment methods. In addition, no studies reported allocation concealment, which would potentially cause bias to occur during functional evaluation. Third, it is uncertain whether all relevant studies were identified. As there were limited studies on the effect of ginsenosides on rats with SCI, only few studies were included.

5. Conclusion

Our study suggests that ginseng extract and ginsenosides significantly improve functional rehabilitation in a rat model of SCI. The probable mechanism by which ginsenosides improve SCI is their antioxidant effects on cells. A higher dosage of ginseng extract and ginsenosides better promotes improvement in the motor function recovery after SCI than a lower dosage of ginseng extract and ginsenosides. However, most included studies were assessed as having low quality. Hence, more high-quality studies are needed to confirm and better explain the effect of ginseng extract and ginsenosides in treating SCI.

Declaration of competing interest

No competing financial interests exist.

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Appendix A. Supplementary data

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