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META-ANALYSIS OF STEM CELL TRANSPLANTATION FOR REFLEX HYPERSENSITIVITY AFTER SPINAL CORD INJURY

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Abstract

Stem cells have been used in novel therapeutic strategies for spinal cord injury (SCI), but the effect of stem cell transplantation on neuropathic pain after SCI is unclear. The current meta-analysis evaluates the effects of stem cell transplantation on neuropathic pain after SCI. We first conducted online searches of PubMed, Web of Science, China Academic Journals Full-text Database, and Wanfang Data for randomized controlled trials that compared stem cell transplantation and vehicle treatments in rodent models of neuropathic pain after SCI. Quality assessment was performed using Cochrane Reviewer's Handbook 5.1.0, and meta-analysis was conducted with RevMan 5.3. Then, we developed a rat model of SCI and transplanted bone marrow mesenchymal stem cells to verify meta-analysis results. Twelve randomized, controlled trials ($n = 354$ total animals) were included in our meta-analysis and divided by subgroups, including species, timing of behavioral measurements, and transplantation time after SCI. Subgroup analysis of these 12 studies indicated that stem cell-treated animals had a higher mechanical reflex threshold than vehicle groups, with a significant difference in both rats and mice. The thermal withdrawal latency showed the same results in mouse subgroups, but not in rat subgroups. In addition, mesenchymal stem cell transplantation was an effective treatment for mechanical, but not thermal reflex hypersensitivity relief in rats. Transplantation showed a positive effect when carried out at 3 or 7 days post-SCI. Stem cell transplantation alleviates mechanical reflex hypersensitivity in rats and mice and thermal reflex hypersensitivity in mice after SCI.

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CONFLICTS OF INTEREST

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

Xuemei Chen and Bohan Xue conceived the study and wrote the manuscript; Bohan Xue, Yuping Li, Peijun Jia, and Xiuhua Ren performed the research; Bohan Xue and Chunhua Song analyzed and interpreted data; Xuemei Chen, Weidong Zang, and Jian Wang revised the manuscript.

Keywords

spinal cord injury; stem cells; neuropathic pain; meta-analysis

INTRODUCTION

Spinal cord injury (SCI) seriously affects human life and is frequently accompanied by neuropathic pain (Hassani-Jirdehi et al., 2015; Stensman, 1994; Westgren and Levi, 1998). The incidence of pain after SCI has been reported to be 77–86% (Donnelly and Eng, 2005). Neuropathic pain is a chronic pain state experienced by approximately 59% of individuals after SCI (Moshourab et al., 2015). SCI-induced neuropathic pain (SCI-NP) can be classified as “at-level” and “below-level” pain. The prevalence of below-level neuropathic pain is in the order of 20–40% (Norrbrink Budh et al., 2003; Siddall et al., 2003; Werhagen et al., 2004). SCI-NP affects not only patients’ physical state, but all aspects of their lives, including work ability, mood, and quality of life; it is also associated with high medical costs (Ferrero et al., 2015; Saulino, 2014). Although many kinds of drugs have been used to treat SCI-NP (e.g., anticonvulsants, antidepressants, and analgesics), even the best treatments have been shown to alleviate only 20–30% of neuropathic pain (Baastrup and Finnerup, 2008). Thus, novel strategies that can inhibit chronic pain after SCI are needed to improve the prognosis and quality of life of patients with SCI-NP.

Recently, use of stem cells has shown great potential in the development of effective therapies for SCI-NP. Studies have revealed that neuronal progenitor and mesenchymal stem cells (MSCs) can be used to repair SCI because they can differentiate into neural cells and secrete growth and neuroprotective factors that promote axonal regeneration and functional recovery as well as recovery of sensory function and pain relief (Ban et al., 2011; Salewski et al., 2015; Watanabe et al., 2015). However, alleviation of pain in SCI-NP patients by stem cell therapies remains contentious. Some researchers have shown that transplantation of MSCs has positive effects and does not induce allodynia (Abrams et al., 2009; Furuya et al., 2009; Ritfeld et al., 2012; Watanabe et al., 2015). On the other hand, Kumagai et al. (2013) reported that naïve MSCs failed to promote functional recovery and reduce hypersensitivity after contusive SCI. Moreover, although some have reported that neural stem cells have beneficial effects, MSC-derived astrocytes and glial-restricted precursor-derived astrocyte transplantation have been limited by graft-induced allodynia in SCI models (Davies et al., 2008; Hofstetter et al., 2005). In the current study, we conducted an online, systematic review of previously published data regarding the effects of stem cell transplantation on SCI-NP. We focused on below-level neuropathic pain. To confirm the results of our meta-analysis, we created our own rat model of SCI-NP, transplanted rat MSCs into the spinal cord, and conducted behavioral tests to monitor development of mechanical and/or thermal reflex hypersensitivity.

EXPERIMENTAL PROCEDURES

Literature search strategy

A comprehensive search was conducted to identify all randomized, controlled trials of stem cell transplantation into SCI animal models that recorded pain threshold as an outcome. We searched PubMed, Web of Science, China Academic Journals Full-text Database, and Wanfang Data databases for original studies and reviews published between 1990 and 2016 with the keywords “stem cells,” “SCI and neuropathic pain,” “SCI,” “cell transplantation,” and “pain;” the language in which articles were published was not restricted. All titles were independently examined by two reviewers, and any report that either reviewer felt was potentially related was initially included.

Inclusion and exclusion criteria

Another two reviewers blinded to experimental goal and re-evaluated all of the initially selected titles; only those titles that both reviewers thought met all inclusion criteria were selected for further study. Any discrepancies between authors were resolved by an arbitrator. Inclusion criteria for selected titles included (1) randomized, controlled animal trials with a parallel design; (2) studies of transplanted stem or progenitor cells of unrestricted cell origin; (3) those investigating rat or mouse models of SCI; (4) reports containing both a vehicle and stem cell transplantation group in which the animals of the vehicle group underwent the same SCI surgery as experimental animals but did not receive stem cells; (5) those that included pain threshold measurements and results. Articles were excluded if (1) the intervention was only stem cell transplantation with any other combined treatment(s); (2) the data of pain threshold results were not numerically variable; (3) the pain threshold was not tested in the hind paw; (4) the stochastic control was of low quality.

Data extraction and risk of bias assessment

The data were extracted independently by two reviewers and rechecked after extraction. Any disagreement during the extraction was discussed and resolved. The content extracted from the selected reports included animal characteristics, interventions, outcome measures, and treatment period. The risk of bias for included trials was assessed according to Cochrane Reviewer's Handbook 5.1.0 (Higgins et al., 2011) and judged using six items: (1) random allocation method; (2) allocation concealment; (3) blind method; (4) incomplete data; (5) selective reporting bias; (6) other bias. Every study was assessed by two independent researchers. Each item was judged as “yes,” “unclear,” or “no,” and all studies were identified as either representing low, unclear, or high bias. Any disagreement in assessment of bias was discussed and resolved.

Creation of contusion SCI rat models

Male Sprague–Dawley rats (250 ± 25 g) were randomly (Cheng et al., 2016; Han et al., 2016) divided into five groups: control (no intervention), sham (laminectomy only), SCI (laminectomy and SCI), vehicle (SCI rats transplanted with Dulbecco's phosphate-buffered saline only; MSC (SCI rats transplanted with MSCs). Each group consisted of 6 rats. Rats were obtained from the experimental animal center of Henan province (China;

SCXK2015-0004). All animals had access to water and food *ad libitum*. For surgeries, 10% chloral hydrate (0.35 mL/100 g) was used to anesthetize animals (Cheng et al., 2015; Wang et al., 2016), and the spinal cord was exposed by laminectomy at the T9–T10 vertebral level. A contusion SCI was produced using an IH-0400 Impactor (PSI, USA) with an impact force of 200 kilodynes. In the sham group, each rat underwent laminectomy only at the T9–T10 vertebral level, with no SCI performed.

MSC culture

MSCs were isolated from the bone marrow of femurs collected from young-adult male Sprague–Dawley rats through gradient centrifugation. The MSCs were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA).

MSC transplantation

MSC transplantation was performed 7 days after SCI. The rats with mechanical withdrawal threshold ≥ 9 were thought to have mechanical reflex hypersensitivity and were used in the subsequent experiments. Rats from each group were anesthetized as described above. Spinal cord of rats in the MSC-treated group was reexposed and then injected with 1×10^6 MSCs in 20 μ L of phosphate-buffered saline in front of and behind the contusion site. To maximize engraftment of all MSCs into the spinal cord, the needle was not disconnected from the spinal cord for 5 min after the injection. Vehicle-treated rats were injected with 20 μ L of Dulbecco's phosphate-buffered saline alone 7 days after SCI.

BEHAVIORAL TESTS

Mechanical reflex hypersensitivity

Mechanical reflex hypersensitivity (Avila-Martin et al., 2015) was recorded at specific time-points after SCI by two independent examiners blinded to the experimental conditions. The rats were placed in a plastic box that was placed on a wire mesh platform. The rats were allowed to move freely and acclimate to the environment. Rats adapted to the environment after approximately 20–30 min. Von Frey filaments (0.4, 0.6, 1, 2, 4, 6, 8, and 15 g) were used to assess the mechanical withdrawal threshold, which was measured with the “up and down” method. Rats underwent two stimulus intervals for 5 min. Baseline was tested 1 day before SCI. The rats that had a mechanical withdrawal threshold ≥ 15 were used for experiments. Tests were administered at 7, 14, 21, and 28 days post-SCI for the SCI, control, and sham groups to explore the effects of SCI on mechanical withdrawal threshold. The test was also administered at 1, 2, 3, 5, and 7 days after MSC transplantation in the MSC and vehicle-treated groups.

Pain-related anxiety behavior

The open-field test was used to evaluate the animals' anxiety after MSC transplantation. Before testing, rats were allowed to adapt to the environment for 2 h. Then they were placed in a 100 $\times$ 100-cm open field surrounded by a black curtain to exclude external cues. The camera, connected to a commercial video-tracking system, was situated above the area in a way that covered the field without casting a shadow. The software divided the area into 25

parts: nine parts in the inner zone (60 × 60 cm) and 16 parts in the outer zone. The chamber was cleaned before and after every test with 75% ethanol. Every rat was recorded and tracked for 5 min, and the software calculated the total time spent in each zone and the total distance moved. Anxiety behavior was confirmed when the animals demonstrated reduced exploration time within the inner zone.

Basso-Beattie-Bresnahan (BBB) score

Hind limb locomotor function was assessed with the 21-point BBB locomotion scale. Rats were tested on days 1, 3, 5, and 7 after SCI and on days 1, 3, 5, and 7 after MSC transplantation. Briefly, the rats were allowed to walk around freely in an open field for 10 min after becoming familiar with the surrounding environment. Hind limb movements were closely observed and independently scored by two observers blinded to the treatment group. The two scores were averaged to provide a final score for each rat.

Statistical analysis

Data analysis was performed with Review Manager 5.3. For continuous outcomes (such as changes of mechanical withdrawal threshold and thermal withdrawal latency), the results were expressed as mean differences with 95% confidence intervals (CI). Because different scales had been applied, the standardized mean difference (SMD) was used. Chi-squared tests and I^2 statistics were used to analyze the heterogeneity among studies. If P was > 0.1 and I^2 was $< 25\%$, there was no significant heterogeneity between studies, and a fixed-effect model was used. If P was < 0.1 and I^2 was $> 25\%$, there was likely substantial heterogeneity. Subgroup analyses were possible because of the variety of animals used in the previous reports, making it necessary to explore heterogeneity. A random-effect model was used to merge heterogeneity that could not be explained. For our animal experiments, behavioral data were expressed as the mean $\pm$ standard error of the mean. All data were statistically analyzed by two-way analysis of variance.

RESULTS

Study selection

After a literature search conducted electronically and manually, the search strategy initially yielded 20 studies that met inclusion criteria (Abrams et al., 2009; Amemori et al., 2013, 2015; Biernaskie et al., 2007; Choi et al., 2013; Dagci et al., 2011; Furuya et al., 2009; Hofstetter et al., 2005; Karimi-Abdolrezaee et al., 2010; Kumagai et al., 2013; Lee et al., 2007; Macias et al., 2006; Nutt et al., 2013; Ritfeld et al., 2012; Salewski et al., 2015; Tao et al., 2013; Urdzikova et al., 2014; Watanabe et al., 2015; Yao et al., 2015; Yousefifard et al., 2016). These articles were retrieved for further assessment of titles, abstracts, and full-texts (if necessary). Of these articles, we excluded seven (Biernaskie et al., 2007; Dagci et al., 2011; Hofstetter et al., 2005; Karimi-Abdolrezaee et al., 2010; Macias et al., 2006; Nutt et al., 2013) that did not measure mechanical withdrawal threshold by the “up and down” von Frey method or measured thermal withdrawal threshold by the tail-flick test. Another was excluded (Abrams et al., 2009) based on exclusion criteria, leaving a total of 12 eligible articles for further assessment (Nutt et al., 2013). Of these, nine reported mechanical

withdrawal threshold, and nine reported thermal withdrawal latency (some studies measured only one or the other, and some measured both; Fig. 1).

Characteristics of selected studies

Table 1 summarizes the characteristics of the 12 randomized, controlled animal trials included in our meta-analysis. Together they represent a total of 354 animals. Of those, 164 underwent SCI surgery, and 190 underwent SCI surgery and stem cell treatment. SCI models included impact injury (7 studies), oppressed injury (3 studies), chemical injury (1 study), and transection injury (1 study) (Table 1).

Risk of bias assessment

All studies had low risk of bias in randomization, allocation method, blinding, and selective reporting. However, incomplete outcomes were not mentioned and therefore associated with a high risk of bias. Other potential sources of bias are listed in Table 2. Overall, the risk of bias was considered to be low in all studies.

Effect of stem cell transplantation on behavioral tests

Because behavioral tests were measured at different times in each study, we divided the reports into two subgroups based on when the tests were administered with respect to stem cell transplantation: 4 weeks and > 4 weeks after transplantation. Then, we assessed any differences between the responses of rat and mouse SCI models.

Mechanical reflex hypersensitivity

Mechanical withdrawal threshold 4 weeks after stem cell transplantation—

Eight studies tested mechanical withdrawal threshold in the first 4 weeks after stem cell transplantation. Of those, six used rat SCI models and two used mouse SCI models. Our results show that mechanical withdrawal threshold was significantly greater in both rats and mice that underwent stem cell transplantation in the first 4 weeks after transplantation than in controls (rat: $SMD = 3.22$, 95% CI = 2.07–4.37, $P < 0.00001$; mouse: $SMD = 3.12$, 95% CI = 2.46–3.77, $P < 0.00001$). The heterogeneity was medium for rats ($I^2 = 76\%$) and low for mice ($I^2 = 0\%$). Overall, the mechanical withdrawal threshold was significantly higher in stem cell-treated than in control animals (rat and mouse) in the first 4 weeks after transplantation ($SMD = 3.09$, 95% CI = 2.30–3.87, $P < 0.00001$), and the heterogeneity was medium ($I^2 = 69\%$). The heterogeneity between rat and mouse subgroups was low ($I^2 = 0\%$; Fig. 2A).

Mechanical withdrawal threshold > 4 weeks after the transplantation—

Six articles tested the mechanical withdrawal threshold > 4 weeks after stem cell transplantation. Of these, five used rat SCI models, and one used a mouse SCI model. Our subgroup analysis showed that the mechanical withdrawal threshold of rats was significantly greater in stem cell-treated than in control animals when measured more than 4 weeks after the transplantation ($SMD = 3.50$, 95% CI = 1.43–5.56, $P = 0.0009$), and the heterogeneity was high ($I^2 = 92\%$). Overall, the mechanical withdrawal threshold was significantly higher in stem cell-treated than in control animals > 4 weeks after transplantation ($SMD = 2.99$, 95%

CI = 1.53–4.44, $P < 0.00001$), and the heterogeneity was high ($I^2 = 90\%$). The heterogeneity between rat and mouse subgroups, however, was low ($I^2 = 38.0\%$; Fig. 2B).

Thermal reflex hypersensitivity

Thermal withdrawal latency in the first 4 weeks after stem cell transplantation

—Eight articles measured thermal withdrawal latency in the first 4 weeks after stem cell transplantation; six of them used rat SCI models, and two used mouse SCI models. We found no significant difference in thermal withdrawal latency between stem cell-treated rats and control rats in the first 4 weeks after transplantation ($P = 0.08$), and the heterogeneity was high ($I^2 = 93\%$). However, the thermal withdrawal latency was significantly higher in stem cell-treated mice than in control mice in this period (SMD = 4.18, 95% CI = 2.89–5.48, $P < 0.00001$). Overall, the thermal withdrawal latency was significantly greater in stem cell-treated than in control animals in the first 4 weeks after stem cell transplantation (SMD = 2.18, 95% CI = 0.60–3.76, $P = 0.007$), and the heterogeneity was high ($I^2 = 96\%$). The heterogeneity between subgroups was also high ($I^2 = 88.9\%$; Fig. 3A). These results indicate that a vast difference exists between rat and mouse subgroups in thermal withdrawal latency during this time period, showing that stem cell transplantation may have a better effect in mice.

Thermal withdrawal latency more than 4 weeks after stem cell transplantation

—Eight articles measured the thermal withdrawal latency > 4 weeks after stem cell transplantation. Seven used rat SCI models, and one used a mouse SCI model. We found no significant difference in thermal withdrawal latency between the stem cell-treated and control rats at more than 4 weeks after transplantation ($P = 0.06$), and the heterogeneity was high ($I^2 = 93\%$). However, the thermal withdrawal latency was significantly greater in stem cell-treated mice than in control mice during this time (SMD = 3.31, 95% CI = 2.51–4.10, $P < 0.00001$). Overall, the thermal withdrawal latency was significantly higher in stem cell-treated than in control animals (both rat and mouse) at > 4 weeks after transplantation (SMD = 1.55, 95% CI = 0.19–2.90, $P = 0.03$), and the heterogeneity was high ($I^2 = 95\%$). The heterogeneity between rat and mouse subgroups was also high ($I^2 = 86.3\%$; Fig. 3B). These results also indicate that stem cell transplantation may have a better effect in mice than in rats.

Effect of MSC transplantation on behavioral tests of SCI models

In the current meta-analysis, seven studies used MSC transplantation. Six of these were rat studies, and one was a mouse study. Therefore, we performed another subgroup analysis based on the time frames described for mechanical and thermal reflex hypersensitivity to determine the effect of MSC-specific transplantation on these behavioral measures.

Mechanical withdrawal threshold in rat SCI models after MSC transplantation

—Four articles measured the mechanical withdrawal threshold during the first 4 weeks after MSC transplantation. Mechanical withdrawal threshold was significantly greater in the MSC-treated SCI rats than in control SCI rats (SMD = 3.12, 95% CI = 1.95–4.28, $P < 0.00001$), with medium heterogeneity ($I^2 = 69\%$; Fig. 4A). Five articles measured mechanical withdrawal threshold at more than 4 weeks after MSC transplantation. Our

analysis of these reports showed that mechanical withdrawal threshold was significantly higher in MSC-treated than in control groups (SMD = 3.38, 95% CI = 1.38–5.38, $P = 0.0009$), with high heterogeneity ($I^2 = 92\%$; Fig. 4B).

Thermal withdrawal latency in rat SCI models after MSC transplantation—Four studies measured thermal withdrawal latency in the first 4 weeks after MSC transplantation, and five measured thermal withdrawal latency at > 4 weeks after MSC. We found no significant difference between MSC-treated and control groups during either time period (4 weeks: $P = 0.08$, > 4 weeks: $P = 0.07$; Fig. 5A, B).

Effect of MSC transplantation time on mechanical reflex hypersensitivity

We next determined the influence of MSC transplantation time after induction of SCI on remission of mechanical reflex hypersensitivity. In the four articles that measured pain behavior in the first 4 weeks after transplantation, mechanical withdrawal threshold was increased when MSCs were transplanted 3 days (SMD = 3.36, 95% CI = 1.76–4.96, $P < 0.0001$) and 7 days (SMD = 2.61, 95% CI = 0.93–4.28, $P = 0.002$) after SCI, and the heterogeneity was high ($I^2 = 85\%$). The overall mechanical withdrawal threshold was significantly greater in the MSC-treated groups than in control groups (SMD = 2.76, 95% CI = 1.38–4.14, $P < 0.0001$), and the heterogeneity was high ($I^2 = 80\%$). The heterogeneity between subgroups was low ($I^2 = 0\%$; Fig. 6A). Of the four articles that measured behavior at more than 4 weeks after transplantation, mechanical withdrawal threshold was also increased when MSCs were transplanted 3 days (SMD = 4.85, 95% CI = 2.76–6.94, $P < 0.00001$) and 7 days (SMD = 4.42, 95% CI = 1.49–7.34, $P = 0.003$) after SCI induction, with high heterogeneity ($I^2 = 92\%$). However, rat SCI models did not show a positive effect when MSCs were transplanted 14 days after SCI ($P = 0.72$; Fig. 6B). These results indicate that transplantation of MSCs within 14 days after SCI can significantly relieve mechanical pain.

MSC transplantation decreases neuropathic pain hypersensitivity to mechanical stimulation after contusion SCI in rats

Based on the results described above, we tested mechanical withdrawal in our contusion SCI rat model before and after transplantation of MSCs. Of the 20 rats that underwent SCI, two failed to show a decrease in mechanical withdrawal threshold within 7 days and were excluded. Among the remaining rats, we found a decrease in the mechanical withdrawal threshold after SCI that stabilized 21 days after injury (Fig. 7A). Additionally, we found a significant increase in the mechanical withdrawal threshold 3 and 5 days after MSC transplantation compared with that in the vehicle-treated groups (Fig. 7B). We observed no significant difference in the distance traveled by sham, MSC-treated, and vehicle-treated groups during the 5-min test session on day 7 after MSC transplantation in open-field test (Fig. 8A). BBB score did not differ significantly between sham, MSC-treated, and vehicle-treated groups. Thus, MSC transplantation did not affect recovery of motor function in the short term (Fig. 8B).

DISCUSSION

Our meta-analysis indicated that stem cell transplantation can effectively alleviate mechanical reflex hypersensitivity in rodent models of SCI, with a remission period duration of more than 4 weeks. Although stem cell (any origin) transplantation effectively induced remission of mechanical and thermal reflex hypersensitivity in mouse SCI models, only mechanical symptoms were relieved in rats; similar results were found in studies of MSC-specific transplantation. Comparison of the influence of different transplantation times after induction of SCI revealed significant improvement in mechanical withdrawal threshold when transplantation was performed at 3 or 7 days after SCI. We confirmed this result in our rat contusion SCI model. All of the results suggest that integration of this approach into current therapeutic strategies may be a new and effective way to treat mechanical hyperalgesia in patients with SCI. This conclusion is further supported by results showing that transplantation of MSCs within 14 days after SCI can significantly alleviate mechanical neuropathic pain. The differing effect of stem cell/MSCT transplantation on thermal reflex hypersensitivity between rats and mice suggests that this approach might not sufficiently treat all neuropathic pain symptoms associated with SCI.

The high heterogeneity in our meta-analysis may be due to different degrees of spinal cord damage, stem cell types/origins, and/or transplantation times after SCI surgery. Moreover, included articles that tested thermal withdrawal latency showed highly different results in rats and mice, suggesting that the mechanism by which stem cells affect thermal reflex hypersensitivity differs between these rodent species. However, some of the studies involving rat models suggested that transplantation of stem cells did not produce any additional hypersensitivity or allodynia (Urdzikova et al., 2014). We believe that the differences between reports involving rat SCI experiments may be due to the difficulty of measuring thermal withdrawal latency. Because movement and function are significantly impaired after SCI, most animals could not put their foot down, which could cause a substantial amount of error in latency measurements. MSCs, as a kind of adult stem cell, can be widely used in the treatment of SCI because of their low immune rejection rate and the advantages of autologous transplantation. In our meta-analysis, we found that MSC transplantation was effective for treating mechanical allodynia, but not thermal allodynia, in rats with SCI. Furthermore, we found the optimal transplantation time to be within 7 days after SCI surgery. Overall, results of our meta-analysis and animal experiments indicate that transplantation of stem cells, particularly MSCs, has a positive influence on SCI-NP.

LIMITATIONS

Although the methodological quality of the 12 included trials had low risk bias, the number of trials examined was small owing to different cell types/origins, cell quantities transfused, and transplantation times after SCI; thus, the heterogeneity between each study was high. Hence, our meta-analysis has a potential risk of bias. In addition, although we included 12 studies in total, no more than 10 each applied to mechanical reflex hypersensitivity or thermal reflex hypersensitivity, individually. Therefore, the publication bias was unsuited for evaluation with Begg's Test or Egger's test and probably influenced the results. Indeed, most of the included studies did not list how many rats were excluded from testing. Consequently,

uncertainty regarding incomplete outcomes also poses a potential risk of bias. At the same time, most continuous outcomes were expressed as baseline and final values, but none of the 12 studies reported changes from baseline, and we extracted some of the data by measuring images provided in the article. It is also possible that SMD results were not indicative of real differences in variability among trial rats, making it difficult to directly interpret the treatment-effect size in our meta-analysis. Twelve trials showed a decrease in pain threshold after SCI, but the timing of those decreases differed. Thus, the severity and/or vertebral level of the SCI might influence neuropathic pain development and treatment decision. Additional studies with larger sample sizes and longer-term outcome measurements are needed to validate the findings described here. Baastrup et al. (2010) reported that ongoing pain was present or sufficient to disturb the animals. They used the open-field test to evaluate the anxiety behavior that represents ongoing pain in animal models of chronic pain and used von Frey filaments to evaluate evoked pain (paw withdrawal). They reported that 97% of SCI animals had spastic syndrome. However studies of experimental SCI pain commonly fail to recognize the presence of spastic syndrome. We suggest that future investigations pay more attention to this condition and include it as an outcome measure.

CONCLUSIONS

Stem cell transplantation alleviated mechanical reflex hypersensitivity in mouse and rat models of SCI and thermal reflex hypersensitivity in mice. Additionally, MSC transplantation significantly reduced neuropathic pain-induced mechanical allodynia after SCI in rats. These results suggest that stem cell transplantation might represent a novel and effective treatment for relief of SCI-NP. However, additional studies with larger sample sizes are necessary to verify these effects.

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Fig. 1.
Flow diagram of online database search.

Fig. 2.

Effect of stem cell transplantation on mechanical withdrawal threshold during the first 4 weeks after transplantation (A) and more than 4 weeks after transplantation (B).

Fig. 3.

Effect of stem cell transplantation on thermal withdrawal latency during the first 4 weeks after transplantation (A) and more than 4 weeks after transplantation (B).

Fig. 4.
 Effect of MSC transplantation on mechanical withdrawal threshold in rat SCI models during the first 4 weeks after transplantation (A) and more than 4 weeks after transplantation (B).

Fig. 5.
 Effect of MSC transplantation on thermal withdrawal latency in rat SCI models during the first 4 weeks after transplantation (A) and more than 4 weeks after transplantation (B).

Fig. 6.

Effect of different MSC transplantation times on mechanical withdrawal threshold in rat SCI models during the first 4 weeks after transplantation (A) and more than 4 weeks after transplantation (B).

Fig. 7.

Mechanical withdrawal threshold before and after MSC transplantation in a rat contusion model of SCI. (A) Mechanical withdrawal threshold after SCI. Data are expressed as mean $\pm$ standard error of the mean ($n = 6$ in each group). Hind paw withdrawal thresholds were significantly decreased in SCI rats compared with those in control and sham groups. ** $P < 0.01$, *** $P < 0.001$ versus sham. (B) Effect of MSC transplantation on mechanical withdrawal threshold. Data are expressed as mean $\pm$ standard error of the mean ($n = 6$ in each group). * $P < 0.05$, ** $P < 0.01$ versus vehicle.

Fig. 8.

The open-field test and BBB score after MSC transplantation in a rat contusion model of SCI. (A) The open-field test after SCI. Data are expressed as mean $\pm$ standard error of the mean ($n = 6$ in each group). There is no significant difference in the distance traveled by sham, MSC-treated, and vehicle-treated groups during the 5-min test session on day 7 after MSC transplantation in open-field test. (B) The BBB score after SCI. Data are expressed as mean $\pm$ standard error of the mean ($n = 6$ in each group). BBB score did not differ significantly between sham, MSC-treated, and vehicle-treated groups.

Table 1

Characteristics of included trials

NO.	Author/Date	Cell	Cells count	Experiment animal	Injury model	Types of Pain	Time for transplantation (after SCI)	The time point of behavior test after transplantation
1	Lee et al. (2007)	MSCs	5×10^5	Rat	II	MWT	7	1d,4d,1w,2w,3w,4w,5w,6w,7w,8w
2	Furuya et al.(2009)	MSCs	2.5×10^6	Rat	II	MWT, TWL	14	6w
3	Ritfeld et al.(2012)	MSCs	1×10^6	Rat	II	MWT, TWL	3	4w,8w
4	Kumagai et al. (2013)	MSCs	4×10^5	Rat	II	MWT, TWL	7	3w,5w
5	Tao et al. (2013)	OPCs	1×10^6	Rat	II	MWT	28	1w,2w,3w,4w
6	Choi et al.(2013)	hATSCs	3×10^6	Mouse	CI	MWT, TWL	0	1w,2w,3w,4w
7	Anemori et al. (2013)	NSCs	5×10^5	Rat	OI	TWL	7	1w,2w,3w,4w,5w,6w,7w,8w
8	Urdzikova et al. (2014)	MSCs	5×10^5	Rat	II	TWL	7	1w,2w,3w,4w,5w,6w,7w,8w
9	Anemori et al. (2015)	NP	5×10^5	Rat	OI	TWL	7	1w,2w,3w,4w,5w,6w,7w,8w
10	Watanabe et al. (2015)	MSCs	2×10^5	Mouse	II	MWT, TWL	3	2w,3,4w,6w
11	Yao et al. (2015)	NSCs	3×10^6	Rat	TI	MWT	0	1w
12	Youseifard et al. (2016)	MSCs	1×10^6	Rat	OI	MWT, TWL	7	1w,2,3w,4w,5w,6w,7

Note: 1. OI = Oppressed injury model; 2. II = Impact injury model; 3. TI = Transection injury model; 4. CI = Chemical injury model 5. NSC = Neural stem cell; 6. MSCs = Mesenchymal stem cells; 7. OPCs = Oligodendroglia cells; 8. hATSCs = human adipose tissue-derived stem cells; 9. NP = neural precursors; 10. MWT = Mechanical withdrawal threshold; 11. TWL = Thermal withdrawal latency.

Table 2

Risk of bias in included trials

Study ID	Sequence generation	Allocation concealment	Blinding Incomplete outcome	Incomplete outcome	Selective reporting	Other bias
Lee et al. (2007)	Randomization (low risk)	Central allocation (low risk)	Double-blind (low risk)	Unclear (high risk)	(low risk)	Only one kind of pain threshold detection method had been used (high risk)
Furuya et al. (2009)	Randomization (low risk)	Central allocation (low risk)	Double-blind (low risk)	Unclear (high risk)	(low risk)	None (low risk)
Ritfeld et al. (2012)	Randomization (low risk)	Central allocation (low risk)	Double-blind (low risk)	Unclear (high risk)	(low risk)	The sample size was Small (high risk)
Kumagai et al. (2013)	Randomization (low risk)	Central allocation (low risk)	Double-blind (low risk)	Unclear (high risk)	(low risk)	None (low risk)
Tao et al. (2013)	Randomization (low risk)	Central allocation (low risk)	Double-blind (low risk)	Unclear (high risk)	(low risk)	Only one kind of pain threshold detection method had been used (high risk)
Choi et al. (2013)	Randomization (low risk)	Central allocation (low risk)	Double-blind (low risk)	Unclear (high risk)	(low risk)	None (low risk)
Anemori et al. (2013)	Randomization (low risk)	Central allocation	Double-blind (low risk)	Unclear (high risk)	(low risk)	The sample size was small and only one kind of pain threshold detection method had been used (high risk)
Urdzikova et al. (2014)	Randomization (low risk)	Central allocation (low risk)	Double-blind (low risk)	Unclear (high risk)	(low risk)	Only one kind of pain threshold detection method had been used (high risk)
Anemori et al. (2015)	Randomization (low risk)	Central allocation (low risk)	Double-blind (low risk)	Unclear (high risk)	(low risk)	Only one kind of pain threshold detection method had been used (high risk)
Watanabe et al. (2015)	Randomization (low risk)	Central allocation (low risk)	Double-blind (low risk)	Unclear (high risk)	(low risk)	None (low risk)
Yao et al. (2015)	Randomization (low risk)	Central allocation (low risk)	Double-blind (low risk)	Unclear (high risk)	(low risk)	The sample size was small and only one kind of pain threshold detection method had been used (high risk)
Youseffard et al. (2016)	Randomization (low risk)	Central allocation (low risk)	Double-blind (low risk)	Unclear (high risk)	(low risk)	None (low risk)