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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. © 2017 Published by Elsevier Ltd on behalf of IBRO.

Key words: 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 (Hassanijirdehi 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-

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Abbreviations: BBB, Basso-Beattie-Bresnahan; CI, confidence intervals; MSCs, mesenchymal stem cells; SCI, spinal cord injury; SCI-NP, SCI-induced neuropathic pain.

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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 $\leq$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 re-exposed and then injected with 1×10^6 MSCs in 20 μL 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 μL 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

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