RESEARCH ARTICLE

Effects of neural stem cell transplantation on the motor function of rats with contusion spinal cord injuries: a meta-analysis

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Abstract

Objective: To judge the efficacies of neural stem cell (NSC) transplantation on functional recovery following contusion spinal cord injuries (SCIs).

Data sources: Studies in which NSCs were transplanted into a clinically relevant, standardized rat model of contusion SCI were identified by searching the PubMed, Embase and Cochrane databases, and the extracted data were analyzed by Stata 14.0.

Data selection: Inclusion criteria were that NSCs were used in *in vivo* animal studies to treat contusion SCIs and that behavioral assessment of locomotor functional recovery was performed using the Basso, Beattie, and Bresnahan lo-comotor rating scale. Exclusion criteria included a follow-up of less than 4 weeks and the lack of control groups.

Outcome measures: The restoration of motor function was assessed by the Basso, Beattie, and Bresnahan locomotor rating scale.

Results: We identified 1756 non-duplicated papers by searching the aforementioned electronic databases, and 30 full-text articles met the inclusion criteria. A total of 37 studies reported in the 30 articles were included in the meta-analysis. The meta-analysis results showed that transplanted NSCs could improve the motor function recovery of rats following contusion SCIs, to a moderate extent (pooled standardized mean difference (SMD) = 0.73; 95% confidence interval (CI): 0.47–1.00; $P < 0.001$). NSCs obtained from different donor species (rat: SMD = 0.74; 95% CI: 0.36–1.13; human: SMD = 0.78; 95% CI: 0.31–1.25), at different donor ages (fetal: SMD = 0.67; 95% CI: 0.43–0.92; adult: SMD = 0.86; 95% CI: 0.50–1.22) and from different origins (brain-derived: SMD = 0.59; 95% CI: 0.27–0.91; spinal cord-derived: SMD = 0.51; 95% CI: 0.22–0.79) had similar efficacies on improved functional recovery; however, adult induced pluripotent stem cell-derived NSCs showed no significant efficacies. Furthermore, the use of higher doses of transplanted NSCs or the administration of immunosuppressive agents did not promote better locomotor function recovery (SMD = 0.45; 95% CI: 0.21–0.70). However, shorter periods between the contusion induction and the NSC transplantation showed slightly higher efficacies (acute: SMD = 1.22; 95% CI: 0.81–1.63; subacute: SMD = 0.75; 95% CI: 0.42–1.09). For chronic injuries, NSC implantation did not significantly improve functional recovery (SMD = 0.25; 95% CI: –0.16 to 0.65).

Conclusion: NSC transplantation alone appears to be a positive yet limited method for the treatment of contusion SCIs.

Key Words: *Basso, Beattie, and Bresnahan locomotor rating scale; cell transplantation; meta-analysis; motor functional recovery; neural regeneration; neural stem cell; neural stem cell transplantation; rat model; spinal contusion; spinal cord injury*

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Introduction

Spinal cord injury (SCI)—one of the most devastating central nervous system (CNS) disorders—can lead to severe neurological disabilities, the most severe forms of which include persistent paraplegia or quadriplegia. The average age at injury has increased from 29 years during the 1970s to 43 years currently, and the annual medical costs per patient are estimated to be over US $100,000 (National Spinal Cord Injury Statistical Center, 2018), which imposes great financial burdens on societies and families.

The primary causes of traumatic SCI include motor vehicle crashes, falls, sports-related injuries, and work-related

injuries (Chen et al., 2013). The primary mechanical trauma results in the laceration of spinal nerve fibers, hemorrhage, and necrosis. Afterwards, a cascade of secondary pathophysiological mechanisms is initiated, including vasospasm, inflammation, demyelination, neurotransmitter and electrolyte disturbances, and the formation of cystic cavities and glial scars. Together, these factors form a microenvironment that is detrimental to recovery from SCI (Stenudd et al., 2015; Fan et al., 2018; Chen et al., 2019). The most common type of SCI in patients is contusion of the spinal cord, which is occurs when vertebral or disc displacement impinges upon the spinal cord (Ramer et al., 2000). Experimental rats suffering from contusion SCIs are similar to human patients with respect to functional, electrophysiological, and morphological outcomes, making rats a suitable animal model for examining functional recovery following SCIs and determining the efficacies of novel strategies for SCI treatment (Metz et al., 2000).

As a result of advances in surgical techniques, medical care, and rehabilitation, the overall prognoses of SCI victims has improved, and patient life spans have been extended to as long as 40 years post-injury; however, in general, current treatments for SCI are not satisfactory (Jain, 2011; Mothe and Tator, 2013). The conventional treatments for SCI include early surgical decompression, the administration of methylprednisolone and neurotrophic drugs, and late re-habilitation. Newly emerging pharmacologic therapies (riluzole, minocycline, fibroblast growth factor, Rho-Rho-associated protein kinase inhibitors, and anti-Nogo antibodies) and nonpharmacologic therapies (hypothermic therapy, cerebrospinal fluid drainage, epidural stimulation, and cell therapy) have been applied in animal experiments or clinical trials (Ahuja et al., 2017; Huang et al., 2019). Cell therapy, especially stem cell transplantation, is regarded as a promising treatment strategy for SCI (Donnelly et al., 2012; García et al., 2019).

Stem cell transplantation has several attractive potential advantages for SCI repair, including the replacement of damaged neurons, the remyelination of spared axons, bridging cysts or cavities, reduced inflammation and gliosis, and the creation of a favorable environment for axonal regeneration (De Feo et al., 2012; Mothe and Tator, 2012). Many studies have demonstrated that neural stem cells (NSCs) have the capacities to differentiate into neural cells and can facilitate motor function recovery after being grafted into the contusion rat model of SCI (Hofstetter et al., 2005; Hwang et al., 2014). In addition, NSCs were have been shown to exert modulatory effects on immune/inflammatory responses (Ottoboni et al., 2015). Therefore, many pioneering works have proposed that NSCs may represent the best cell type for neural repair in victims with SCI (Cheng et al., 2012; Amemori et al., 2013). Conversely, several studies have reported that functional recovery was not examined when NSCs were used as SCI treatments in rats (Macias et al., 2006; Yan et al., 2007).

Currently, there is limited data available regarding whether the use of transplanted NSCs are beneficial for functional recovery in a clinically relevant, standardized rat model of contusion SCI. Hence, this study aimed to perform a me-ta-analysis to determine the efficacies of NSC transplantation on functional recovery following SCI.

Data and Methods

Search strategy

Electronic databases (PubMed, Embase, and the Cochrane databases) were searched through the end of September 2018. The search strategy was based on the terms "neural stem cell", "neural precursor cell" and "neural progenitor cell" combined with terms relevant to "spinal cord injury". Additionally, we conducted a manual search of previous review articles and the reference lists of all related articles to identify additional studies. Only studies published in English were retrieved. Two reviewers independently screened the titles and abstracts of the articles obtained from the electronic databases for inclusion. If the abstract was related to this study, we read the full-text and determined whether the article was eligible for inclusion. Disagreements between the reviewers were resolved by mutual discussion.

Inclusion and exclusion criteria

Inclusion criteria were as follows: 1) rats were used as the subjects of *in vivo* SCI animal studies; 2) a clinically relevant, standardized animal model of contusion was used; and 3) studies included behavioral assessments of locomotor functional recovery that adopted the Basso, Beattie, and Bresnahan locomotor rating scale. Exclusion criteria were as follows: 1) studies used a follow-up period of less than 4 weeks, which was regarded as the minimum amount of time required for the observation of cell therapy effects on locomotor functional recovery; 2) studies lacked control groups (saline-treated or vehicle-treated groups); and 3) review articles, meta-analyses, editorials and commentaries.

Quality assessment

According to recommendations provided in studies by Antonic et al. (2013), Hassannejad et al. (2016), and Yousefifard et al. (2016), we designed a checklist to evaluate the quality of every included study. The 17-item checklist was as follows: 1) publication in a peer-reviewed journal; 2) description of animals' age/weight; 3) description of animals' strain; 4) description of location of contusion SCI; 5) description of severity of contusion SCI; 6) description of number of animals per group; 7) random allocation of animals to specific groups; 8) allocation concealment; 9) use of ap-propriate tests and methods to answer the primary objective(s) of study; 10) blindness of assessors; 11) description of exclusion criteria for animals in each treatment group; 12) description of statistical analysis; 13) description of the control groups; 14) description of compliance with regulations and ethical guidelines for animal studies; 15) statement describing temperature control; 16) bladder expression; and 17) statement of any potential conflicts of interest. Two reviewers independently evaluated all included studies and assigned each study a score of good, fair or poor. Disa-greements between reviewers were settled by consensus.

Data extraction and synthesis

Two reviewers independently investigated all included articles and related animal studies. Data extraction was con-ducted by reviewers blinded to the journal, author and organization. We designed a checklist based on PRISMA

statement guidelines to record relevant data (Moher et al., 2009) (**Additional file 1**). The data collected were as follows: 1) animal characteristics (strain, gender, and weight); 2) SCI animal model details (location of injury and severity of injury); 3) transplanted NSC features (origin, donor species, donor age, and derivation); 4) details of transplanted NSCs (cell dose, graft route, graft type, and intervention time); 5) follow-up period; 6) use of immunosuppressant, neuroprotective anesthetic, and neuroprotective antibiotic factors; 7) blinding of assessors; and 8) outcomes of motor function recovery 4 weeks after cell transplantation. In cases of disagreements between the two reviewers, a third reviewer extracted the data.

Data concerning motor function outcomes were recorded as the mean, the standard deviation of the mean, the sample size of each group, and the standard error of the mean. When data were presented as charts, we used a method recommended by Sistrom and Mergo (2000), and ImageJ software (version 1.51, NIH, 2017; https://imagej.nih.gov/ij/notes.html) was used to extract the data. When information was not provided, we attempted to contact the authors via e-mail to seek clarification.

Statistical analysis

Relevant data were entered into the Stata statistical software (version 14.0; Stata Corp., College Station, TX, USA) as the mean and the standard deviation of the mean. Using the Hedges' g, a standardized mean difference (*SMD*) was calculated, with a confidence interval of 95% (95% *CI*), for each individual comparison, and then a pooled effect size was expressed. Heterogeneity was assessed using chi-square and I^2 tests. For the chi-square test, a *P*-value of 0.1 or less was considered to indicate the existence of significant heterogeneity. Based on the Cochrane Handbook for Systematic Reviews of Interventions (Deeks et al., 2011), the thresholds used during the interpretation of I^2 were as follows: 1) 0–40%: might not be important; 2) 30–60%: may represent moderate heterogeneity; 3) 50–90%: may represent substantial heterogeneity; and 4) 75–100%: represents considerable heterogeneity. The fixed effect model was used for homogenous studies, and random effect model results were used if there was evidence for moderate, substantial, or considerable heterogeneity between studies because they reported more conservative effects than the fixed effect model (Higgins and Thompson, 2002). If heterogeneity existed, we performed subgroup analyses to attempt to identify the sources of heterogeneity. Subgroup analyses were conducted based on animal gender, rat strain (Sprague-Dawley [SD] and non-SD), animal weight, severity of injury (moderate and severe), donor species (rat, mouse, and human), donor age (fetal and adult), cell derivation (brain and spinal cord), cell type (wild-type and induced pluripotent stem cell [iPSC]-derived), cell dose, graft route (intra-spinal and intrathecal), graft type (allogeneic and xenogeneic), intervention time (acute, subacute, and chronic), follow-up period (less than 8 weeks, equal to 8 weeks and more than 8 weeks), immunosuppressive agent, neuroprotective anesthetic, neuroprotective antibiotic and blindness of assessors. Publication bias was assessed by the Egger's and Begg's tests (Begg and Mazumdar, 1994; Egger et al., 1997). Meta-analyses were performed only if the data were reported by no less than three studies.

Results

Characteristics of the included studies

We identified 1756 non-duplicated papers by searching the aforementioned electronic databases. Of these, 150 poten-tially relevant articles were screened, and 30 full-text articles met the inclusion criteria. A total of 37 studies were included in the meta-analysis (Watanabe et al., 2004; Hofstetter et al., 2005; Macias et al., 2006; Neuhuber et al., 2008; Hwang et al., 2009, 2014, 2016; Kim et al., 2009; Lee et al., 2009; Maeda et al., 2009; Lebedev et al., 2010; Xu et al., 2010, 2011, 2012; Alexanian et al., 2011; Mitsui et al., 2011; Cheng et al., 2012; Hu et al., 2012; Niapour et al., 2012; Liu et al., 2013; Yang et al., 2013; Emgård et al., 2014; Hong et al., 2014; Ormond et al., 2014; Wu et al., 2015; Zhang and Shen, 2015; Jin et al., 2016; López-Serrano et al., 2016; Ye et al., 2016; Stewart et al., 2017). A diagram summarizing the study selection process is shown in **Figure 1**. The detailed information for the data included in the final meta-analysis is summarized in **Table 1**.

Extracted data from 784 rats (412 in the transplantation group and 372 in the control group) were pooled and analyzed together. Evaluations were performed in 603 female and 181 male experimental animals, which included 567 SD and 180 non-SD rats. In 33 studies, cell transplantation was performed using wild-type NSCs, and iPSC-derived NSCs (iPSC-NSCs) were applied to four animal experiments. The doses of transplanted NSCs ranged from 1.11×10^5 to 1.45×10^7 cells per kilograms of body weight. The graft type was allogeneic in 21 animal experiments. The mean time interval between contusion and transplantation was 11.1 ± 16.3 days (ranging from 0 to 91 days). In six experiments, transplantation was conducted immediately after contusion induction (acute phase); in 26 experiments, this gap was 3–13 days apart (subacute phase); and in five experiments, the procedure occurred greater than or equal to two weeks after induction (chronic phase). The administration of immunosuppressive agents during cell transplantation was performed in 16 experiments. In seven experiments, the assessors did not follow the principle of blindness when assessing motor function recovery outcomes.

Quality assessments of the included studies are presented in **Table 2**. Extracted data from the included studies regard-

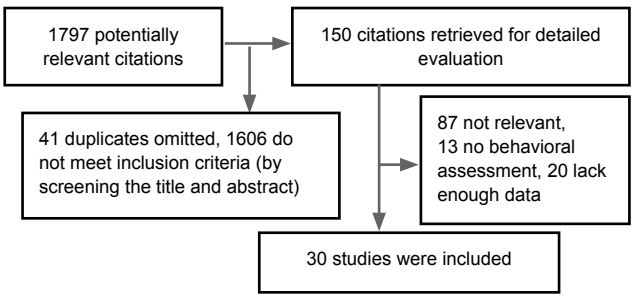

Figure 1 Flow diagram showing the selection of studies for the meta-analysis.

Table 1 Characteristics of the studies included in the meta-analysis

Study	Strain/gender/weight/number	Location of injury/ severity of injury	Origin/cell source/dose/route/type/intervention time/follow up	Anesthetic/immunosuppressant/ antibiotic/blinding
Stewart et al. (2017)	SD/female/250–300 g/27	T9/severe	Wild-type/rat, fetus, brain/3.0×10^5/intra-spinal/ allogeneic/9 d/6 wk	Isoflurane/no/enrofloxacin/yes
Hwang et al. (2016)	SD/male/200–220 g/14	T12/moderate	Wild-type/mouse, fetus, ESC/1.0×10^6/intrathecal/ xenogeneic/21 d/7 wk	Isoflurane/cyclosporine/ampicillin, sulbactam/yes
Jin et al. (2016)	–/female/225–250 g/19	T10/severe	Wild-type/rat, fetus, spinal cord/1.0×10^6/ intra-spinal/allogeneic/91 d/8 wk	Xylazine, acepromazine maleate, ketamine/cyclosporine/cefazolin/yes
López-Serrano et al. (2016)	SD/female/250–300 g/26	T8–9/moderate	iPSC-derived/human, adult, dermal fibroblast/1.0×106/intra-spinal/xenogeneic/ 0, 7 d/60, 53 d	Ketamine, seduxen/FK506/amoxicillin/no
Ye et al. (2016)	SD/female/200–250 g/25	T10/severe	Wild-type/monkey, fetus, brain/2.5×10^4/ intra-spinal/xenogeneic/0 d/10 wk	Chloral hydrate/no/amoxicillin/yes
Wu et al. (2015)	SD/male/200–250 g/10	T8–9/severe	Wild-type/rat, fetus, brain/4.0×10^5/intrathecal/ allogeneic/0 d/4 wk	Ketamine/no/no/yes
Zhang and Shen (2015)	SD/male/190–210 g/16	T10/moderate	Wild-type/rat, fetus, brain/1.0×10^5/ cerebroventricular/allogeneic/3 d/4 wk	Pentobarbital sodium/no/penicillin/no
Emgård et al. (2014)	Athymic mice/female/ 170–200 g/18	T13/moderate	Wild-type/human, fetus, spinal cord/1.0×10^5/ intra-spinal/xenogeneic/9 d/17 wk	Fentanyl citrate, fluanisone, midazolam/ immunodeficient/trimethoprim sulfa/yes
Hong et al. (2014)	SD/female/230–250 g/42	T9/severe	Wild-type, iPSC-derived/mouse, fetus, brain; mouse, fetus, fibroblast/1.0×10^6/intra-spinal/ xenogeneic/9 d/12 wk	Isoflurane/cyclosporine/cefotiam/yes
Hwang et al. (2014)	SD/female/250–300 g/16	T9/moderate	Wild-type/rat, fetus, spinal cord/5.0×10^5/ intra-spinal/allogeneic/7 d/7 wk	No/no/no/yes
Ormond et al. (2014)	SD/female/200–250 g/34	T9–10/moderate, severe	Wild-type/rat, adult, brain/1.0×10^6/intra-spinal/ allogeneic/7 d/6 wk	Pentobarbital sodium/no/amoxicillin/yes
Liu et al. (2013)	SD/female/250–300 g/60	T10/severe	Wild-type/rat, fetus, brain/4.0×10^6/intrathecal/ allogeneic/0, 7, 28 d/12, 11, 8 wk	Pentobarbital sodium/no/cefazolin/yes
Yang et al. (2013)	LE/male/250–300 g/10	T9–10/moderate	Wild-type/pig, fetus, ESC/1.0×10^6/intra-spinal/ xenogeneic/7 d/24 wk	No
Cheng et al. (2012)	LE/female/250–300 g/12	T10/severe	Wild-type/human, fetus, brain/5×10^5/intrathecal/ xenogeneic/0 d/7 wk	Isoflurane/no/cefazolin/yes
Hu et al. (2012)	SD/female/250–300 g/24	T9/severe	Wild-type/rat, fetus, spinal cord/4.0×10^5/ intra-spinal/allogeneic/9 d/6 wk	Pentobarbital sodium/no/ chloramphenicol/yes
Niapour et al. (2012)	Wistar rat /male/250–300 g/16	T9–10/severe	Wild-type/human, fetus, ESC/0.5×10^6/ intra-spinal/xenogeneic/7 d/5 wk	Ketamine, xylazine/cyclosporine/ gentamycin/yes
Xu et al. (2012)	Wistar rat /male/200–250 g/60	T9–10/severe	iPSC-derived/rat, adult, bone marrow/1.0×10^6/tail vein/allogeneic/7 d/5 wk	Pentobarbital sodium/no/no/no
Alexanian et al. (2011)	SD/female/200–250 g/20	T8/severe	Wild-type/human, fetus, neural tissue/1.0×10^5/ intra-spinal/xenogeneic/8 d/7 wk	Ketamine, medetomidine/prograf/ enrofloxacin/yes
Mitsui et al. (2011)	SD/female/225–250 g/17	T8–9/Severe	Wild-type/rat, fetus, spinal cord/0.5×10^6/ intra-spinal/allogeneic/9 d/8 wk	Xylazine, acepromazine maleate, ketamine/cyclosporine/ampicillin/yes
Xu et al. (2011)	SD/female/200g/18	T9/moderate	Wild-type/rat, fetus, brain/5×10^5/intra-spinal/ allogeneic/56 d/8 wk	Pentobarbital sodium/no/no/yes
Lebedev et al. (2010)	Wistar rat /male/300–350 g/40	T9/moderate	Wild-type/human, adult, olfactory epithelium/1.5×10^6/intrathecal/xenogeneic/ 0 d/8 wk	Ketamine, seduxen/no/no/no
Xu et al. (2010)	Wistar rat /female/ 210–230 g/27	T9–10/severe	Wild-type/rat, fetus, brain/6.0×10^5/intra-spinal/ allogeneic/14 d/12 mon	Pentobarbital sodium/no/no/yes
Hwang et al. (2009)	SD/female/250–300 g/23	T9–10/moderate	Wild-type/human, fetus, brain/2×10^5/intra-spinal/ xenogeneic/7 d/6 wk	Chloral hydrate/cyclosporine/no/yes
Kim et al. (2009)	SD/female/200–250 g/16	T9/moderate	Wild-type/human, fetus, brain/2×10^5/intra-spinal/ xenogeneic/7 d/5 wk	Chloral hydrate/cyclosporine/no/yes
Lee et al. (2009)	SD/female/200–250 g/20	T9/moderate	Wild-type/human, fetus, brain/2×10^5/intra-spinal/ xenogeneic/7 d/6 wk	Chloral hydrate/cyclosporine/no/yes
Maeda et al. (2009)	Wistar rat /male/250–300 g/15	T10/severe	Wild-type/rat, fetus, brain/1×105/intra-spinal/ allogeneic/7 d/6 wk	Isoflurane/no/no/no
Neuhuber et al. (2008)	SD/female/–/40	T8–9/severe	Wild-type/rat, fetus, spinal cord/2.0×10^6, 1.0×10^6/ intrathecal, intra-spinal/allogeneic/9 d/8 wk	Xylazine, acepromazine maleate, ketamine/cyclosporine/no/yes
Macias et al. (2006)	SD/female/200–250 g/13	T8/severe	Wild-type/mouse, newborn, brain/1×10^5/ intra-spinal/xenogeneic/8 d/4 wk	Ketamine, medetomidine/Prograf/ Enrofloxacin/yes
Hofstetter et al. (2005)	SD/female/250 g/48	T8–9/moderate	Wild-type/rat, adult, spinal cord/5×10^5/ intra-spinal/allogeneic/7 d/9 wk	Halothane/no/no/yes
Watanabe et al. (2004)	SD/female/230–250 g/58	T10/severe	Wild-type/rat, fetus, brain and spinal cord/5×10^5/ intra-spinal/allogeneic/9 d/12 wk	No/no/no/yes

ESC: Embryonic stem cell; follow up: the period of time after cell transplantation; iPSC-derived: induced pluripotent stem cell-derived neural stem cells; LE: Long Evans rats; SD: Sprague-Dawley rats; T: thoracic level of spinal cord; Wild-type: wild-type neural stem cells.

Table 2 An overview of the quality assessment scores assigned to the included studies, using a 17-item checklist

Study	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Quality assessment results
Stewart et al. (2017)	+	+	+	+	+	+		+	+	+		+	+	+		+	+	Good
Hwang et al. (2016)	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	Good
Jin et al. (2016)	+	+		+	+	+		+	+	+		+	+	+	+	+		Good
López-Serrano et al. (2016)	+	+	+	+	+	+			+			+	+	+	+	+	+	Good
Ye et al. (2016)	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	Good
Wu et al. (2015)	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	Good
Zhang and Shen (2015)	+	+	+	+	+	+	+		+			+	+	+		+	+	Good
Emgård et al. (2014)	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		Good
Hong et al. (2014)	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		Good
Hwang et al. (2014)	+	+	+	+	+	+	+	+	+	+		+	+			+		Good
Ormond et al. (2014)	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	Good
Liu et al. (2013)	+	+	+	+	+	+	+	+	+	+		+	+	+	+		+	Good
Yang et al. (2013)	+	+	+	+	+	+			+			+	+	+			+	Good
Cheng et al. (2012)	+	+	+	+	+	+	+	+	+	+		+	+	+		+		Good
Hu et al. (2012)	+	+	+	+	+	+		+	+	+		+	+	+	+	+		Good
Niapour et al. (2012)	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	Good
Xu et al. (2012)	+	+	+	+	+	+			+			+	+	+				Good
Alexanian et al. (2011)	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	Good
Mitsui et al. (2011)	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+		Good
Xu et al. (2011)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		Good
Lebedev et al. (2010)	+	+	+	+	+	+			+			+	+			+		Good
Xu et al. (2010)	+	+	+	+	+	+	+	+	+	+		+	+	+		+		Good
Hwang et al. (2009)	+	+	+	+	+	+	+	+	+	+		+	+			+		Good
Kim et al. (2009)	+	+	+	+	+	+	+	+	+	+		+	+	+		+		Good
Lee et al. (2009)	+	+	+	+	+	+		+	+	+		+	+	+		+		Good
Maeda et al. (2009)	+	+	+	+	+	+			+			+	+	+				Good
Neuhuber et al. (2008)	+		+	+	+	+		+	+	+		+	+	+	+	+	+	Good
Macias et al. (2006)	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+		Good
Hofstetter et al. (2005)	+	+	+	+	+	+	+	+	+	+		+	+	+			+	Good
Watanabe et al. (2004)	+	+	+	+	+	+		+	+	+		+	+	+				Good

According to recommendations provided in studies by Antonic et al. (2013), Hassannejad et al. (2016), and Yousefifard et al. (2016), we designed a checklist to evaluate the quality of all included studies, which included the following 17 items. 1: Publication in a peer-reviewed journal; 2: description of animals' age/weight; 3: description of animals' strain; 4: description of location of the contusion SCI; 5: description of the severity of the contusion SCI; 6: number of animals per group; 7: random allocation of animals to specific groups; 8: allocation concealment; 9: use of appropriate tests and methods to answer the primary objective(s) of study; 10: blindness of assessors; 11: description of exclusion criteria for each treatment group; 12: description of statistical analysis; 13: description of the control groups; 14: description of compliance with regulations and ethical guidelines for animal studies; 15: statement describing temperature control; 16: bladder expression; 17: statement of any potential conflicts of interest.

ing motor function recovery are listed in **Table 3**, including the mean, the standard deviation of the mean, the sample sizes of each group, and the standard error of the mean.

Results of the meta-analysis

In this meta-analysis, 30 articles, which included 37 studies, evaluated the efficacies of NSC transplantations on the restoration of motor function in rats following SCIs. Due to the existence of substantial heterogeneity (I^2 = 65.4%; χ^2 = 104.04, P < 0.001), we calculated the pooled SMD by utilizing the random effect model. The transplantation of NSCs led to the statistically significant improvement of locomotor functional recovery in rats after contusion SCIs (pooled SMD = 0.73; 95% CI: 0.47–1.00; P < 0.001). The relevant findings are shown in **Figure 2**, in the form of a forest plot. Publication bias was not observed in this part of the study (Egger test: Coefficient = 1.88; 95% CI: –0.78 to 4.55; P = 0.16. Begg test: P = 0.64). The results of the subgroup analyses are presented in **Table 4**. Transplantations of NSCs in male rats (SMD = 1.99; 95% CI: 1.28–2.69) resulted in better functional recovery compared with trans-plantations in female rats (SMD = 0.44; 95% CI: 0.27–0.60). The results also showed better motor function recovery in non-SD rats (SMD = 1.84; 95% CI: 1.19–2.50) compared with SD rats (SMD = 0.44; 95% CI: 0.27–0.62). Similar cell efficacies were observed between rat (SMD = 0.74; 95% CI: 0.36–1.13) and human (SMD = 0.78; 95% CI: 0.31–1.25) NSCs, between fetal (SMD = 0.67; 95% CI: 0.43–0.92) and adult (SMD = 0.86; 95% CI: 0.50–1.22) NSCs, and between brain-derived (SMD = 0.59; 95% CI: 0.27–0.91) and spinal cord-derived (SMD = 0.51; 95% CI: 0.22–0.79) NSCs. iPSC-NSC transplantations did not significantly improve motor function recovery (P = 0.154). Likewise, higher doses (SMD = 0.62; 95% CI: 0.19–1.05) of engrafted NSCs did not promote better locomotor functional recovery than lower doses (SMD = 0.88; 95% CI: 0.52–1.23). When NSCs were transplanted during the acute (SMD = 1.22; 95% CI: 0.81–1.63) or subacute (SMD = 0.75; 95% CI: 0.42–1.09) phases, the transplantations were more efficacious than when NSCs were transplanted during the chronic phase (SMD = 0.25; 95% CI: –0.16–0.65).

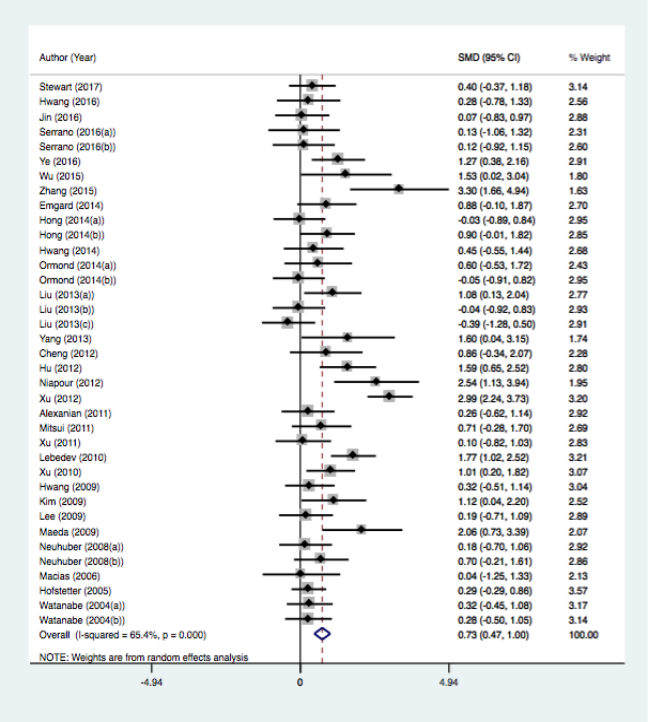

Figure 2 Efficacy of neural stem cell transplantations on the restoration of motor function after spinal cord injury.
The transplantation of neural stem cells significantly improved the locomotor function of rats with spinal cord contusion injuries (pooled standardized mean difference (SMD) = 0.73; 95% confidence interval (CI): 0.47–1.00; $P < 0.001$).

Additionally, transplantations performed without immunosuppressants (SMD = 0.93; 95% CI: 0.52–1.34) had similar and slightly higher efficacies than transplantations performed with the administration of immunosuppressants (SMD = 0.45; 95% CI: 0.21–0.70). Similarly, transplantations with and without the administration of anesthetics and antibiotics with neuroprotective effects showed similar locomotor functional recovery outcomes in rats (anesthetic: SMD = 0.77 *versus* SMD = 0.71; antibiotic: SMD = 0.48 *versus* SMD = 0.88).

Discussion

The present study reviewed data gathered from a clinically relevant, standardized contusion SCI rat model to evaluate the efficacies of NSC transplantations on functional recovery in rats using a quantitative approach. According to the interpretation of effect sizes described in the Cochrane Handbook for Systematic Reviews of Interventions (Schunemann et al., 2011), the pooled SMD represented a moderate effect. The results of the quantitative analysis indicated that engrafted NSCs could improve the motor function recovery in rats to a moderate extent. Previous studies have shown that transplanted NSCs can survive, proliferate and differentiate in injured spinal cords; however, the majority of these transplanted NSCs differentiated toward astro- or oligodendroglial linages, and NSCs rarely differentiated into neuronal phenotypes (Vroemen et al., 2003; Xu et al., 2010). Motor function recovery may benefit more from the secretion of trophic factors and cytokines with neuroprotective and immunomodulatory effects released by engrafted NSCs (Kumamaru et al., 2012; Liang et al., 2014), which can sustain the survival of host cells and support local axonal sprouting (Hofstetter et al., 2005). Furthermore, experimental studies have reported that the combination of two or more strategies [e.g., NSCs + novel tissue engineering materials (Hosseini et al., 2016), other types of cells (Niapour et al., 2012; Stewart et al., 2017), or the overexpression of specific transcription factors that facilitate neural repair (Hofstetter et al., 2005)] resulted in superior functional improvements versus NSC transplantation alone. Hence, the efficacy of treating SCIs in rats using NSC transplantations alone was positive but limited.

Earlier studies provided evidence that females had a gender-related advantage with regards to motor function recovery and the preservation of spared spinal cord tissue following contusive SCI in adult rats (Datto et al., 2015), which may be the result of neuroprotective effects provided by sex hormones, such as estrogen and progesterone (Brotfain et al., 2016). Likewise, SD rats recovered faster and achieved greater functional recovery than Long Evans and Wistar rats in the contusion model of SCI, suggesting that genetic factors may be involved in functional recovery (Mills et al., 2001). However, no direct experimental evidences existed that demonstrated the influence of rat gender or strain on functional recovery following contusion SCIs when receiving identical NSC transplantations. Therefore, we conducted subgroup analyses based on rat gender and strain. Intriguingly, the results showed that Long Evans and Wistar rats and male rats had experienced increased restoration of motor function compared with their counterparts. The preliminary interpretation of theses result was that qualitative differences exist in the cellular immune response to contusion SCIs among different strains of rat (Popovich et al., 1997), and transplanted NSCs demonstrate modulatory effects on immune/inflammatory responses (Kumamaru et al., 2012). Determining how the inherent characteristics of different rat strains and the immunomodulatory effects of NSCs influence functional recovery after SCI requires further investigation. The observation of the increased efficacy of transplanted NSCs in male rats is difficult to rationalize, and more studies and larger sample sizes are necessary to prove that this observed effect is real. In addition, these results indicated that variables such as gender and strain could significantly influence the outcomes of studies using animal models of SCI (Hassannejad et al., 2016).

Both fetal and adult brains, as well as the spinal cord, in rodents and humans contain NSCs that can self-renew and be induced to generate enriched populations of neuronal or glial progenitors, both *in vitro* and *in vivo* (Mothe and Tator, 2013). In the past several decades, fetal or adult NSCs from different species (rodent or human) and different derivation origins (brain or spinal cord) have been adopted in numerous animal experiments (Watanabe et al., 2004; Ormond et al., 2014) and several clinical trials (clinicaltrials.gov identifier Number: NCT01321333, NCT01772810) to promote the repair of SCI. However, the optimal source of NSCs for the treatment of SCI remains under debate (Tetzlaff et al., 2011). Therefore, we conducted subgroup analyses based on donor species, donor age, and stem cell derivation, and the

Table 3 Data regarding motor function recovery gathered from the included studies

	Transplantation group				Control group			
Study	Mean	SDM	*N*	SEM	Mean	SDM	*N*	SEM
Stewart et al. (2017)	12.68	2.41	11	0.73	11.61	2.68	16	0.67
Hwang et al. (2016)	13.61	3.75	7	1.42	12.43	4.16	7	1.57
Jin et al. (2016)	10.45	3.11	10	0.98	10.28	1.32	9	0.44
López-Serrano et al. (2016a)	10.42	1.87	5	0.84	10.21	1.13	6	0.46
López-Serrano et al. (2016b)	10.51	1.3	9	0.43	10.36	1.06	6	0.43
Ye et al. (2016)	8.84	0.96	15	0.25	7.57	0.98	10	0.31
Wu et al. (2015)	15.66	1.18	5	0.53	13.64	1.2	5	0.54
Zhang and Shen (2015)	16.3	0.54	8	0.19	14	0.76	8	0.25
Emgård et al. (2014)	15.37	2.41	10	0.76	13.58	1.01	8	0.36
Hong et al. (2014a)	8.2	2.46	12	0.71	8.26	2.01	9	0.67
Hong et al. (2014b)	10.2	2.1	12	0.61	8.26	2.01	9	0.67
Hwang et al. (2014)	11.81	4.72	8	1.67	9.94	2.97	8	1.05
Ormond et al. (2014a)	12.15	3.69	7	1.39	9.34	5.06	6	2.06
Ormond et al. (2014b)	4.84	6.61	9	2.2	5.06	1.75	12	0.51
Liu et al. (2013a)	9.6	1.57	10	0.5	8.2	0.77	10	0.24
Liu et al. (2013b)	8.15	0.88	10	0.28	8.2	1.28	10	0.4
Liu et al. (2013c)	8.1	0.97	10	0.31	8.55	1.23	10	0.39
Yang et al. (2013)	10.35	2.1	6	0.86	3.94	5.27	4	2.63
Cheng et al. (2012)	8.83	6.82	6	2.79	4	2.59	6	1.06
Hu et al. (2012)	12.51	0.6	12	0.17	11.5	0.63	12	0.18
Niapour et al. (2012)	10.76	1.73	8	0.61	5.97	1.84	8	0.65
Xu et al. (2012)	12.8	1.1	30	0.2	10	0.71	30	0.13
Alexanian et al. (2011)	10.48	1.49	10	0.47	9.62	4.23	10	1.34
Mitsui et al. (2011)	8.73	1.3	8	0.46	7.2	2.52	9	0.84
Xu et al. (2011)	10.7	1.54	9	0.51	10.55	1.29	9	0.43
Lebedev et al. (2010)	12.59	1.06	23	0.22	10.97	0.61	17	0.15
Xu et al. (2010)	9.84	0.72	14	0.19	9.28	0.19	13	0.05
Hwang et al. (2009)	11.34	1.32	12	0.38	10.89	1.4	11	0.42
Kim et al. (2009)	10.24	0.89	8	0.32	9.38	0.51	8	0.18
Lee et al. (2009)	11.24	2.2	12	0.63	10.7	3.35	8	1.18
Maeda et al. (2009)	8	0.85	8	0.3	6.35	0.62	7	0.24
Neuhuber et al. (2008a)	7.81	3.69	10	1.17	7.23	2.39	10	0.76
Neuhuber et al. (2008b)	8.74	1.69	10	0.54	7.23	2.39	10	0.76
Macias et al. (2006)	8.9	14.19	10	4.49	8.34	5.85	3	3.38
Hofstetter et al. (2005)	12.73	7.05	20	1.58	11.21	3.38	28	0.64
Watanabe et al. (2004a)	8.99	9.78	20	2.19	6.13	6.34	10	2.01
Watanabe et al. (2004b)	9.19	12.53	18	2.95	6.13	6.34	10	2.01

N: Sample size of per group; SDM: standard deviation of mean; SEM: standard error of mean. Data were entered into the Stata 14.0 statistical software to calculate pooled effect sizes.

results indicated that there were no significant differences in the efficacies for functional recovery between rat and human NSCs, between fetal and adult NSCs, or between brain-derived and spinal cord-derived NSCs.

The use of the NSCs procured from fetal or embryonic human origin tissues is restricted due to ethical issues and the insufficiency of autologous cell sources (Mothe and Tator, 2013), whereas NSCs originating from adult tissues can be used an alternative cell type for stem cell therapy, without ethical concerns. iPSC-NSCs that are produced indirectly from autologous cell sources via reprogramming can also avoid the immune rejection response. Although several publications have shown functional regeneration following iPSC-NSC transplantations in contusive SCI cases (Nori et al., 2011; Hong et al., 2014), some studies reported no significant therapeutic effects (López-Serrano et al., 2016). The subgroup analysis performed in this study showed that iPSC-NSC transplantations did not significantly promote motor function recovery following SCI. This subgroup of iPSC-NSCs included four studies, three of which used adult somatic tissue-derived iPSC-NSCs. These cells display significant resistance to differentiation (Tsuji et al., 2011) and are difficult to propagate for abundant cell transplantation (Mothe and Tator, 2013). Furthermore, the risk of tumor for-mation further limited the use of adult tissue-derived iPSC-NSCs for SCI implantation (Tsuji et al., 2011).

The optimal dose of transplanted NSCs also remains under debate. A review by Yousefifard et al. (2016) found that higher doses resulted in better restoration of motor function than lower doses, due to the increased chance of NSC survival at higher doses, leading to the formation of efficient connections with the injured tissue. In this meta-analysis, the median dose of NSCs per kilogram of animal body weight was 2.08×10^6, which was chosen as the cut-off point for the subgroup

Table 4 Subgroup analyses of the effects of neural stem cells on locomotor recovery

Characteristic	N*	Model	P (I^2)†	SMD‡ (95% CI)	P
Gender					
Male	8	REM	0.004 (66.4%)	1.99 (1.28–2.69)	< 0.001
Female	29	FEM	0.481 (0.0%)	0.44 (0.27–0.60)	< 0.001
		Overall significance test among subgroups			< 0.001
Strain					
SD	28	FEM	0.084 (28.2%)	0.44 (0.27–0.62)	< 0.001
Non-SD	7	REM	0.011 (63.8%)	1.84 (1.19–2.50)	< 0.001
		Overall significance test among subgroups			< 0.001
Severity of injury					
Moderate	14	REM	0.009 (53.3%)	0.68 (0.34–1.07)	< 0.001
Severe	23	REM	< 0.001 (71.0%)	0.75 (0.39–1.12)	< 0.001
		Overall significance test among subgroups			0.874
Cell source (donor species)					
Rat	21	REM	< 0.001 (73.8%)	0.74 (0.36–1.13)	< 0.001
Mouse	4	FEM	0.502 (0.0%)	0.33 (–0.17 to 0.83)	0.198
Human	10	REM	0.018 (55.1%)	0.78 (0.31–1.25)	0.001
		Overall significance test among subgroups			0.984
Cell source (donor age)					
Fetal	29	REM	0.004 (45.7%)	0.67 (0.43–0.92)	< 0.001
Adult	7	REM	< 0.001 (87.5%)	0.86 (0.50–1.22)	0.001
		Overall significance test among subgroups			0.59
Cell source (derivation)					
Brain	19	REM	0.007 (50.3%)	0.59 (0.27–0.91)	< 0.001
Spinal cord	9	FEM	0.401 (4.0%)	0.51 (0.22–0.79)	< 0.001
		Overall significance test among subgroups			0.912
Cell type					
Wild-type	33	REM	0.001 (50.4%)	0.66 (0.42–0.89)	< 0.001
Induced pluripotent stem cell-derived	4	REM	< 0.001 (89.5%)	1.07 (–0.40 to 2.54)	0.154
		Overall significance test among subgroups			0.26
Dose					
≥ 2.08×10^6 cell/kg	18	REM	< 0.001 (75.0%)	0.62 (0.19–1.05)	0.005
< 2.08×10^6 cell/kg	17	REM	0.005 (53.5%)	0.88 (0.52–1.23)	< 0.001
		Overall significance test among subgroups			0.336
Graft route					
Intra-spinal	27	FEM	0.100 (26.9%)	0.55 (0.37–0.72)	< 0.001
Intrathecal	8	REM	0.005 (65.1%)	0.63 (0.04–1.21)	0.036
		Overall significance test among subgroups			0.883
Graft type					
Allogeneic	21	REM	<0.001 (73.8%)	0.74 (0.36–1.13)	< 0.001
Xenogeneic	16	REM	0.024 (45.7%)	0.73 (0.38–1.07)	< 0.001
		Overall significance test among subgroups			0.998
Intervention time					
Acute	6	FEM	0.317 (15.1%)	1.22 (0.81–1.63)	< 0.001
Subacute	26	REM	< 0.001 (69.3%)	0.75 (0.42–1.09)	< 0.001
Chronic	5	FEM	0.225 (29.5%)	0.25 (–0.16 to 0.65)	0.233
		Overall significance test among subgroups			0.066
Follow-up period					
≥ 8 weeks	19	REM	0.031 (41.4%)	0.54 (0.28–0.81)	< 0.001
< 8 weeks	18	REM	< 0.001 (74.9%)	0.97 (0.49–1.46)	< 0.001
		Overall significance test among subgroups			0.15
Immunosuppressive agent					
Yes	16	FEM	0.373 (7.1%)	0.45 (0.21–0.70)	< 0.001
No	21	REM	< 0.001 (75.6%)	0.93 (0.52–1.34)	< 0.001
		Overall significance test among subgroups			0.126
Neuroprotective anesthetic					
Yes	28	REM	< 0.001 (71.9%)	0.77 (0.43–1.11)	< 0.001
No	5	FEM	0.360 (8.1%)	0.71 (0.30–1.13)	0.001
		Overall significance test among subgroups			0.966
Neuroprotective antibiotic					
Yes	15	REM	0.020 (47.8%)	0.48 (0.13–0.84)	0.008
No	6	REM	0.031 (59.5%)	0.88 (0.24–1.53)	0.008
		Overall significance test among subgroups			0.326
Blindness of assessors					
Yes	30	FEM	0.139 (22.3%)	0.49 (0.32–0.66)	< 0.001
No	7	REM	< 0.001 (80.5%)	1.69 (0.75–2.62)	< 0.001
		Overall significance test among subgroups			0.001

Acute: Zero to two days after injury; Chronic: more than or equal to fourteen days after injury; CI: confidence interval; FEM: fixed effect model; Non-SD: Long Evans and Wistar rats; REM: random effect model; SD: Sprague-Dawley rats; SMD: standardized mean difference; Subacute: three to thirteen days after injury. *Number of studies per group. † Heterogeneity among studies. ‡ Standardized mean difference.

analysis. The subgroup analysis found that higher doses did not promote better functional improvement. Kumamaru et al. (2012) demonstrated that higher doses made spinal contusion injuries worse by eliciting a robust inflammatory reaction, resulting in a negative effect on functional recovery. Moreover, Piltti et al. (2015) showed that the expansion of donor NSCs was inversely regulated by target niche parameters and/or the initial donor cell density, indicating that the SCI niche may have a limited capacity to accommodate donor cells. The results described above indicated that an appropriate range for the number of transplanted cells exists for SCI repair. During clinical trials, transplantation protocols are diverse, and the dose of transplanted cells varies considerably (Aboody et al., 2011), which has hampered the establishment of an optimal treatment protocol for SCI. Hence, further defining the optimum cell dose is necessary to maximize functional regeneration.

Motor function recovery after NSC transplantations can be influenced by intervention time. The meta-analysis showed that transplantations during the acute or subacute stages were associated with better results than transplantations during the chronic stage. During the chronic phase of SCI, secondary injury mechanisms become stabilized and the formation of a glial scar acts as both a physical and chemical barrier, hampering the survival and integration of transplanted NSCs in the injured spinal cord (Mothe and Tator, 2013). NSC transplantations are considered to be more effective during the subacute phase than during the acute phase because inflammatory responses induced by acute SCIs can create a detrimental environment that affects the survival of NSCs (Parr et al., 2007). Intriguingly, the meta-analysis revealed no significant differences in the efficacies of transplantations between the acute phase and the subacute phase ($P = 0.066$), and treatments during the acute phase had similar and slightly higher efficacies than those during the subacute phase. Kumamaru et al. (2012) reported that, although acutely injected NSCs were not yet differentiated into replacements for damaged neural cells, they could exert neuroprotective effects by enhancing the secretion of neurohumoral substances, including brain-derived neurotrophic factor and insulin-like growth factor 1. These factors play critical roles in the prevention of neuronal apoptosis via the nuclear factor-κB pathway (Kaltschmidt et al., 2005) and block the activation of the mitochondrial apoptotic pathway associated with secondary damage cascades (Sofroniew et al., 2001). Furthermore, Kumamaru et al. (2012) reported that the number of infiltrating inflammatory cells and the expression levels of pro-inflammatory cytokines and extrinsic apoptotic-cascade mediators induced by inflammation did not differ significantly from those observed in controls during the acute phase of SCI, suggesting that NSC transplantation can exert immunomodulatory effects by ameliorating pathological changes and/or stimulating beneficial inflammatory responses (Assinck et al., 2017).

Early studies reported that immunosuppressants could exert inhibitory effects on graft rejection responses and facilitative effects on cell survival, in addition to playing neuroprotective and neurotrophic roles that enhanced the rate and length of axon regeneration and the degree of axon myelination (Lü et al., 2010; Sevc et al., 2013). The findings showed that the use of immunosuppressants had similar and slightly reduced efficacies compared with transplantations without the administration of immunosuppressants, which was extremely difficult to rationalize. The most plausible explanations were as follows. First, the CNS has been traditionally regarded as an immunologically privileged site (Niederkorn, 2006; Galea et al., 2007), and NSCs possess a low immunogenic nature due to the lack of the major histocompatibility complex and the expression and survival of certain co-stimulatory signaling molecules in non-immune-privileged sites (Hori et al., 2003; Capetian et al., 2011). Second, for the 21 studies included in this meta-analysis that did not use immunosuppressants, the most common graft type was allogeneic cell transplantation, and the animal strains were the same between the donor cells and the recipient animal, which could minimize immunological incompatibility issues (Hwang et al., 2014). Third, immunosuppressive drugs can have inhibitory effects on wound healing during SCI (Park et al., 2013).

Several drugs, such as anesthetics (isoflurane, ketamine, and propofol) and antibiotics (amoxicillin and cefazolin), have been shown to have various degrees of neuroprotective effects. General anesthetics penetrate into the CNS and may exert neuroprotective effects by reducing neuronal activity and metabolism and may protect neurons from the damaging effects of free radical generation and extracellular glutamate accumulation, which closely resemble the pathomechanism of SCI (Jain, 2011). The neuroprotective mechanism of antibiotics may be associated with the ability of β-lactam antibiotics to stimulate the expression of glutamate transporter-1, which can prevent the neurotoxicity elicited by extracellular glutamate accumulation (Rothstein et al., 2005). However, the subgroup analyses showed that similar effects were observed regardless of whether neuroprotective anesthetics/antibiotics were used. The temporary or short-term use of these neuroprotective agents may not provide practical neuroprotective effects and functional improvements.

Several clinical trials have reported that NSC transplantations into the lesions of SCI patients could be safe, but the effects of NSC transplantations on functional recovery have not yet been proven (Curtis et al., 2018). Notably, for NSCs derived from human fetal tissues, there are ethical concerns and risks of tumor formation. Additionally, *in vitro* adult human NSCs are challenging to expand, which can make obtaining enough cell yield for transplantations difficult, and autologous sources of adult human NSCs are insufficient and result in the requirement for immunosuppression. The findings of the present meta-analysis showed that NSCs obtained from different donor species, different donor ages and different derivation origins had similar efficacies for the improvement of functional recovery, whereas adult iPSC-NSCs showed no significant efficacies. Furthermore, higher doses of transplanted NSCs and the administration of immunosuppressive agents did not promote better locomotor functional recovery. However, a shorter period between the contusion induction and the NSC transplantations showed slightly higher cell efficacies. The meta-analysis provided insight into some aspects of transplantation, such as the influences of NSC sources and doses, the time of grafting, and immunosuppressive administration, which can be used

to design effective clinical trials. In the future, more clinical trials and larger sample sizes are necessary to demonstrate the efficacies of NSC transplantation in humans and to elucidate the relevant mechanisms underlying the effects of NSC transplantation into the sites of human SCIs.

In this meta-analysis, a series of measures were taken to improve the quality of this study. First, we performed an extended search of electronic databases, in combination with a manual search and, where necessary, contacted the authors to fill gaps to facilitate the inclusion of the maximum number of articles. Second, we calculated SMDs using the Hedges' g to compare results across the articles and to correct for biases caused by small sample sizes. Last, the absence of publication biases also strengthened the quality of this study. Nevertheless, some limitations existed in this study. One limitation was the substantial heterogeneity among the included studies, which was addressed by performing subgroup analyses. A second limitation was that some of the original articles did not report the blinding status of assessors. Last, the functional recovery in a young population with SCI may be better than that of an older population. Because most of the included studies examined young adult rats, the data were not sufficient to determine the efficacies of NSC transplantations older populations, and determining whether differences exist in the efficacies of NSC transplantations between younger and older populations require further study.

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2-4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4-5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4-5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6-7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6-7

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	9-10
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7-8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	10
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9-10
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9-10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11-16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	16
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	16
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	17

From: Moher D, Liberati A, Tetzlaff J, Altman DG; The PRISMA Group (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 6:e1000097.

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