Mesenchymal Stem Cell-Conditioned Medium Promotes Functional Recovery Following Spinal Cord Injury: A Systematic Review and Meta-analysis

Arash Sarveazad[1)2)], Amirmohammad Toloui[3)], Aida Moarrefzadeh[4)], Hanieh Ghasemian Nafchi[3)5)], Arian Madani Neishaboori[3)] and Mahmoud Yousefifard[3)]

1) Colorectal Research Center, Iran University of Medical Sciences, Tehran, Iran
2) Nursing Care Research Center, Iran University of Medical Sciences, Tehran, Iran
3) Physiology Research Center, Iran University of Medical Sciences, Tehran, Iran
4) Department of Psychology, Ahar Branch, Islamic Azad University, Ahar, Iran
5) Department of Physiology, Iran University of Medical Sciences, Tehran, Iran

Abstract:

Background: Considering the limitations of cell therapy, in case of adequate treatment efficacy, conditioned media (CM) may be a desirable alternative to cell therapy. Hence, the present systematic review and meta-analysis aims to evaluate the efficacy of mesenchymal stem cell-derived conditioned media (MSC-CM) in movement resolution following spinal cord injury (SCI) in animal models.

Methods: A comprehensive search in the databases of Medline, Scopus, Web of Science, and Embase was completed until the end of March 2021. Animal studies that evaluate the efficacy of MSC-CM on movement resolution following SCI were defined as the inclusion criteria. Lack of an SCI-untreated group, CM derived from a source other than MSC, not assessing motor function, failure to report CM administered dose, a follow-up period of less than 4 weeks, duplicates, and review articles were counted as the exclusion criteria. Final results are presented as overall standardized mean difference (SMD) with a 95% confidence interval (CI).

Results: From the 361 nonduplicate articles, data from 11 articles were entered into the present meta-analysis. The analyses showed that MSC-CM administration in SCI animal models promotes motor recovery (SMD=2.32; 95% CI: 1.55, 3.09; p<0.0001). Subgroup analysis was performed because of the noticeable heterogeneity between the studies (I^2=80.97%, p< 0.0001), depicting that antibiotic administration, delivery amount, delivery type, and follow-up time were the possible sources of heterogeneity. Moreover, multiple meta-regression demonstrated that in cases of delivery amount of more than 120 μL, the efficacy of MSC-CM administration in motor recovery is more than that of delivery amount of less than 120 μL (regression coefficient=3.30; 95% CI: 0.72, 5.89; p=0.019).

Conclusions: Based on the results of the present study, it can be concluded that MSC-CM administration in SCI models improves motor recovery. The efficacy of this treatment strategy significantly increases at doses higher than 120 μL.

Keywords:

Conditioned media, Mesenchymal stem cells, Motor function, Spinal cord injury, Systematic review

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Introduction

Spinal cord injury (SCI) is one of the most devastating nervous injuries, most commonly occurring in the young population and causing life-span disabilities in patients. Approximately 78% of SCI patients suffer from moderate to severe pains. SCI and its complications impose major direct and indirect financial costs, both on the affected families and health systems, in such a way that the annual treatment cost of these patients is estimated to be approximately $26270[1)].

Following SCI, a cascade of reactions occurs in the in-

Corresponding author: Mahmoud Yousefifard, yousefifard.m@iums.ac.ir
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jured area, all of which lead to tissue destruction and the loss between cellular connections. This nerve destruction causes dissociation between upper-level neurons and lower-level ones. Thus, it seems that the disabling symptoms will not resolve until the injured area is repaired and efficient connections develop between the neurons proximal to the injured area and the ones distal to the area[2,3]. Therefore, researchers seek new approaches to be taken to promote the reconstruction of the damaged cells and tissue. Nowadays, it is widely hypothesized that cell transplantation can be an appropriate candidate in the treatment of SCI. Some researchers hypothesized that cell transplantation to the injured spinal cord may restore new neural connections, attenuating the debilitating symptoms[4]. Various pieces of evidence exist, indicating that mesenchymal stem cells (MSCs) transplantation may improve motor function following an injury to the central nervous system (CNS)[5-7]. MSCs secrete several cellular factors, promoting a desirable environment for the neural tissue to regenerate itself. It is known that only 1% of the transplanted MSCs survive after 1 week following transplantation[8-10]. Therefore, it can be concluded that their efficacy is mostly attributed to their paracrine responses and the various growth factors that they secrete[11,12]. Consequently, the excreted solution following the paracrine activity of these cells is called conditioned media, which effectively enhances neuronal tissue survival after the injury through activating several molecular pathways, such as the phosphoinositide 3-kinase/Akt pathway[13-15]. Consequently, in the case of MSCs derived conditioned medium (MSC-CM) transplantation being as effective as MSCs transplantation in the treatment of SCI, MSC-CM may be a desirable alternative for MSCs in the treatment of SCI, since it does not have the limitations of MSCs.

Nevertheless, there exist contradictions between the current studies over the subject. For instance, in 2018, Asadigolshan et al. demonstrated that MSC-CM is ineffective in motor recovery following SCI[16], whereas in 2017, Gu et al. reported considerable motor recovery following the MSC-CM treatment in SCI animal models[17]. Similarly, countless other studies are available, all of which present different results for the treatment. Thus, a consensus over the matter is yet to be achieved, and the factors causing this diversity should be identified. Thus, the present systematic review and meta-analysis aims to investigate the efficacy of MSC-CM transplantation in the treatment motor deteriorations in animal models of SCI.

Method

Study design and search strategy

The present study was designed to investigate the efficacy of MSC-CM treatment on motor recovery following SCI in animal models. For this purpose, a comprehensive search was conducted on the electronic databases of Medline (through PubMed), Scopus, Web of Science, and Embase completed until the end of March 2021. The search strategy was designed using keywords related to MSCs, conditioned media, and SCI. The search strategy in the Medline database is presented in Supplementary table 1. Besides the systematic search, a manual search in the related articles' reference and through search engines Google and Google Scholar was performed.

Selection criteria

The definition of PICO in the present is as follows: Problem or study population (P): animal models of SCI; intervention or index (I): MSC-CM administration; Comparison (C): comparison between the treated and nontreated SCI animals; and Outcome (O): motor recovery evaluation based on standard tests.

Accordingly, the inclusion criteria were studies being conducted to investigate the efficacy of CM administration in SCI animal models. Studies that lacked a control nontreated SCI group, studies in which the source of CM was non-MSC stem cells, studies that transplanted MSCs instead of CM, studies that did not report the desired outcome or the CM preparation method, studies in which the follow-up period was less than 4 weeks (since motor recovery requires a time of at least four weeks following the injury), duplicate studies, and review studies were excluded.

Data collection

Two independent reviewers performed article screening and summarized the data. First, titles and abstracts of the searched articles were screened and related articles were selected. Then, full texts of the potentially related articles were reviewed, and based on the inclusion and exclusion criteria, included articles were selected. Afterward, data, including the study design, animals' characteristics (weight, sex, species, and number of animals in each group), injury mechanism, injury site, interval time between injury and MSC-CM administration, type of the stem cell used (autograft, allograft, and xenograft), source of the MSC-CM, number of the MSCs in the medium, the medium type used for cell culture, dosage and administration route of the CM, number of administrations, use of immunosuppressants, and antibiotics and follow-up period were extracted from the selected articles. Regarding the follow-up period, the eventual follow-up time was extracted from the articles. Moreover, since most of the selected studies reported their findings within their graphs, Sistorm and Mergo's method was adopted to gather the mean and standard deviation (SD) of the articles[18]. In all of the reported steps, any disagreements were resolved using a third reviewer's opinion.

Quality assessment of the articles

The risk of bias was assessed using the proposed method by Hassannejad et al[19]. The method is a checklist used for quality assessment of animal studies on SCI. This tool encompasses 15 questions regarding the characteristics of the studied animals, injury method, animal care, sampling

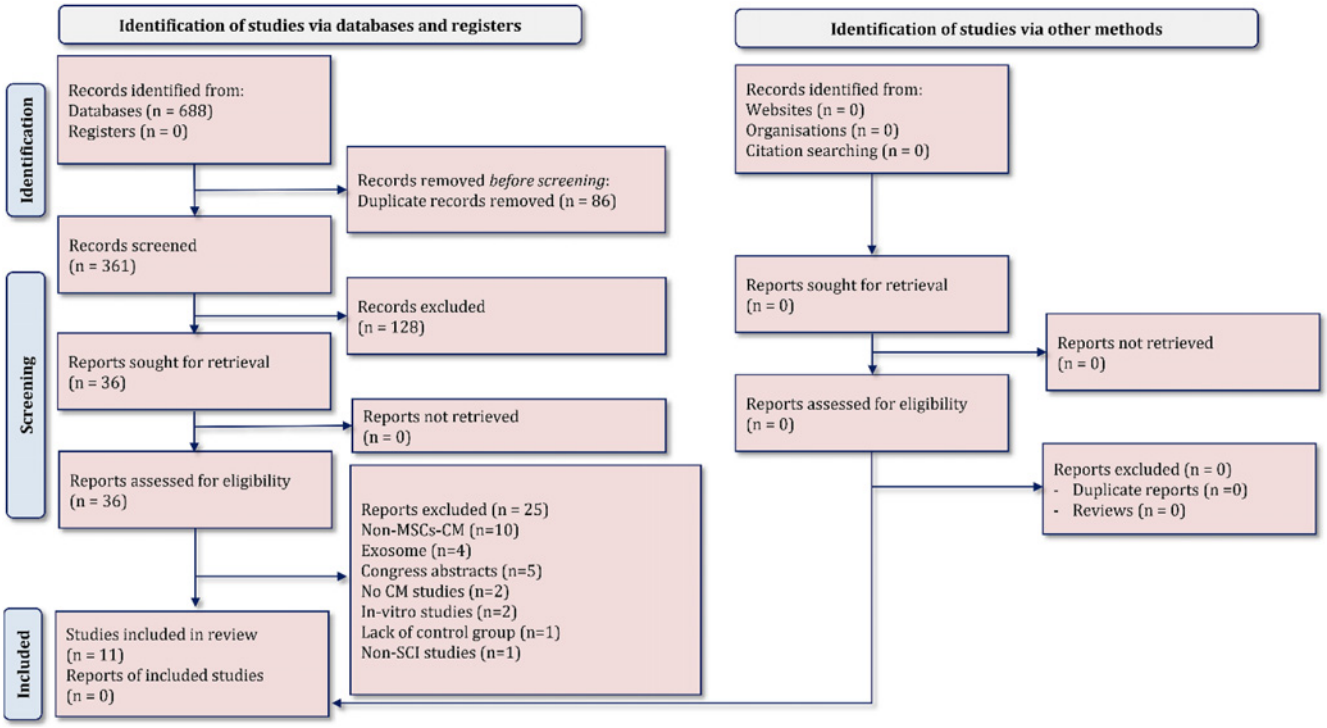

Figure 1. PRISMA flow diagram of the present meta-analysis.

method, definitions of the nontreated control group and treatment group, statistical analyses performed, the number of the excluded animals, and the reason for their exclusion. Each reviewer was assigned an independent risk of bias evaluation of the articles' presented data and responded low risk, high risk, and not reported to the checklist's questions. Similar to the data collection, any disagreements were resolved using a third reviewer's opinion.

In cases of fatal errors, the study was considered as having a high risk of bias, and in case of having a low risk of bias in all of the items, the study was considered as having a low risk of bias. Furthermore, in cases of not having fatal errors but at least one question was responded to as high risk of bias or at least two were responded to as not reported, the study would have been considered as a concern in the risk of bias evaluation. In the present study, fatal errors included lack of blinding of the assessor, not using the standard test for assessment of locomotion, and not reporting the severity and spinal level of SCI.

Statistical analysis

Data were analyzed using STATA 14.0 statistical program. All of the included studies were classified on the basis of the extent of motor recovery. Data were entered into the program as means and SDs. Since a number of the included studies reported standard errors (SEs) instead of SD, SD was calculated by multiplying SE to the square root of the number of animals in the group. Moreover, since the methodologies differed (for instance, in terms of administration route and the administration dosage of MSC-CM) in the original studies, based on the performed pilot study, diversity among the included articles was anticipated, and the analyses were performed using random effect model. Also, heterogeneity was evaluated using the Chi-square test and I^2 statistics. In cases of heterogeneity, a subgroup analysis was performed to identify the source of the heterogeneity. Eventually, the study results were pooled together, and the overall effect size was reported. This effect size was reported as standardized mean difference (SMD) and 95% confidence interval (CI). Noteworthily, meta-analyses were performed in cases that were reported in at least three studies.

To identify the independent factors, multivariate meta-regression was performed to investigate the effect of methodology differences (for instance in the transplantation route and the administered dose of MSC-CM) on the motor recovery. For this purpose, the variables identified in the subgroup analysis as being potential sources of heterogeneity were included in this multivariate model. Furthermore, to evaluate the individual study effect, a sensitivity analysis based on the leave-one-out approach was performed. Additionally, a sensitivity analysis based on the overall risk of bias was performed. To evaluate the publication bias, a funnel plot and Egger's test were adopted[20].

Results

Characteristics of the included studies

The initial search resulted in 361 nonduplicated articles. After the initial screening, full texts of 36 articles were studied, and finally, 11 articles were entered into the present meta-analysis[14-17,21-27] (Fig. 1). The reasons for exclusion of the articles were the use of CM derived from sources other than MSCs (n=10), exosome administration instead of CM

Table 1. Characteristics of Included Studies.

Study	Animals	n SCI, n treat	SCI model and location	Injury to treatment (days)	Cell source, type of graft	Medium	Immunosuppressive, antibiotic	Delivery amount (μL) and type	Follow-up (weeks)
Asadi-Golshan, 2018[16]	Male, Rat, SD, 250–280	10, 10	Compression, Moderate, T7	0	Dental Pulp, Xenogeneic	DMEM	No, Yes	3, Single-dose, In situ	6
Borhani-Haghighi, 2020[21]	Male, Rat, SD, 250–280	6, 6	Compression, Moderate, T7	2	Breast milk stem cell, Xenogeneic	DMEM	NR, No	3, Single-dose, IT	6
Cantinieaux, 2013[14]	Female, Rat, Wistar, 200	10, 10	Contusion, Severe, T11	0	BMSCs, Allogenic	DMEM	NR, No	10, Single-dose, IT	6
Chen, 2019[22]	Female, Rat, SD, 250–270	14, 26	Transection, Severe, T8	0	BMSCs, Xenogeneic	DMEM and NRLM	NR, Yes	120, Single-dose, IT	8
Chudickova, 2019[23]	Male, Rat, Wistar, 250–300	20, 15	Compression, Moderate, T8	7	UCMSCs, Xenogeneic	CCM	NR, Yes	50, Single-dose, IT	9
Cizkova, 2018[15]	Male, Rat, Wistar, 300–320	4, 6	Compression, Moderate, T8–T9	0	BMSCs, Allogenic	DMEM	NR, No	120, Single-dose, IT	10
Gu, 2017[17]	Male, Rat, SD, 250–300	18, 36	Contusion, Moderate, T10	0	OEC, Allogenic	DMEM	NR, Yes	42000 and 84000, Multidose, IP	6
Kanekiyo, 2018[24]	Female, Rat, SD, 200	10, 10	Contusion, Severe, T8–T9	0	BMSCs, Allogenic	DMEM	NR, Yes	1400, Single-dose, Intraventricular	4
Khoshsirat, 2018[25]	Female, Rat, Wistar, 180–200	8, 8	Contusion, Moderate, T9–T10	0	BMSCs, Allogenic	DMEM	No, Yes	NR, Single-dose, IP	12
Tsai, 2019[26]	Female, Rat, SD, 250–350	9, 9	Contusion, Moderate, T10	0	BMSCs, Allogenic	DMEM	NR, No	450, Single-dose, IV	6
Yeng, 2016[27]	Male, Rat, SD, 206–230	8, 8	Contusion, Severe, T8–T10	0	UCMSCs, Xenogeneic	DMEM	NR, Yes	500, Single-dose, IV	1

BMSCs: Bone marrow mesenchymal stem cell; CCM: Complete culture medium; DMEM: Dulbecco's Modified Eagle's medium; ECGM: Endothelial cell growth medium; IP: Intraperitoneal; IT: Intrathecal; IV: Intravenous; NR: Not reported; NRLM: neural regeneration laboratory medium; OEC: Olfactory ensheathing cell; SD: Sprague-Dawley; UCMSCs: Umbilical cord mesenchymal stem cells

(n=4), no CM administration (n=2) in vitro studies (n=2), lacking a nontreated SCI control group (n=1), non-SCI study (n=1), and not reporting the required variables due to the study being presented in a congress (n=5). Worth mentioning is that the authors of congress published studies were contacted, and their names with relevant keywords were searched in electronic databases so that no articles would be neglected. However, no result regarding the last five excluded articles was found.

All of the included articles were performed on rats. The injury method was contusion in six articles, compression in four articles, and transection in one article. The severity of injury was moderate in seven articles and severe in the other four. The Basso, Beattie, and Bresnahan test was used in all of the included studies for motor function evaluation. The score ranges from 0 (no movement) to 21 (no impairment). Only data gathered using this test was extracted from the studies since it was the only test used in all of the studies. Nine studies administered the CM immediately after the injury, one study started treatment one day after the injury, and one other study started their treatment seven days post-SCI. The MSCs were from the bone marrow in six articles, umbilical cord in two studies, dental pulp in one study, and olfactory ensheathing cells and breast milk stem cells in one other study. Only two studies reported that they had not used immunosuppressive agents, and the other nine articles did not report whether or not they had used immunosuppressive. Seven articles administered antibiotics, and four other articles did not. Ten studies applied a single-dose treatment regimen. The single-dose injections varied between 3 and

Table 2. Risk of Bias Assessment of Included Studies.

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Item 11	Item 12	Item 13	Item 14	Item 15	Overall
Asadi-Golshan, 2018	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	Low
Borhani-Haghighi, 2020	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NR	✓	✓	NR	High
Cantinieaux, 2013	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NR	Low
Chen, 2019	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NR	Low
Chudickova, 2019	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NR	Low
Cizkova, 2018	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NR	✓	✓	NR	High
Gu, 2017	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NR	Low
Kaneliyo, 2018	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NR	✓	✓	✓	NR	Some concern
Khoshsirat, 2018	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NR	Low
Tsai, 2019	✓	✓	✓	✓	NR	✓	✓	✓	✓	✓	✓	✓	✓	✓	NR	Some concern
Yeng, 2016	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NR	Low

1. Species; 2. Using appropriate tests; 3. Severity of injury; 4. Level of injury; 5. Age/weight; 6. Number of animals per group; 7. Designation of strain; 8. Definition of control; 9. Description of statistical analysis; 10. Regulation and ethics; 11. Bladder expression; 12. Blindness of assessor; 13. Genetic background; 14. Method of allocation to treatments; 15. Description of the reasons to exclude animals from the experiment during the study (attrition).
✓: Low risk of bias; NR: Not reported

1400 μL, and the multidose varied between 42000 and 84000 μL. Transplantation route was intrathecal in five studies, intraperitoneal in two studies, intravenous in two studies, intraventricular in one study, and in situ in one other study. The follow-up period ranged from 1 to 12 weeks (1 week in one study, 4 weeks in one study, 6 weeks in five studies, 8 weeks in one study, 9 weeks in one study, 10 weeks in one study, and 12 weeks in one other study) (Table 1).

Risk of bias

Items of species, using appropriate tests, severity of injury, level of injury, designation of strain, definition of control, description of statistical analysis, regulation and ethics, genetic background, and method of allocation to treatments were at low risk in all of the studies. Moreover, age/weight, bladder expression, and the number of the animals per group were each not reported in one study. Finally, a description of the reasons to exclude animals from the experiment during the study was reported in only one study (Table 2).

Since two studies did not report the blinding status of the assessor, they had fatal errors and were considered as having a high risk of bias. Furthermore, two studies were scored to have some concern status and seven studies were classified as having a low risk of bias.

Meta-analysis

Motor recovery

Data from all of the 11 articles were evaluated in this section. Analyses demonstrated that MSC-CM administration in SCI animal models improves motor recovery (SMD=2.32; 95% CI: 1.55, 3.09); nevertheless, considerable heterogeneity was observed among the studies (I^2=80.97%, p<0.0001) (Fig. 2). Consequently, the studies were classified on the basis of the severity of SCI, cell source, antibiotic administration, treatment protocol, delivery type, medium type, type of graft, delivery amount, and follow-up duration, and separate analyses were performed for each of the subgroups (Table 3). Subgroup analysis showed that heterogeneity among the studies in which antibiotic was not administered (I^2=0.00%; p=0.494) was lower than that of the other studies (I^2= 87.12%; p<0.001). Moreover, nearly all of the studies that used an intrathecal route were homogenous (I^2=32.47%; p= 0.205). Eventually, calculations show that the possible sources of heterogeneity were the delivery amount (I^2= 56.46%; p=0.032) and the follow-up duration (I^2=67.14%; p =0.022).

Meta-regression

Multivariate meta-regression showed that among the studied factors, only the administered dosage of MSC-CM affects the efficacy of the treatment. In other words, in doses higher than 120 μL, the efficacy of MSC-CM treatment is significantly higher than that of doses lower than 120 μL (coefficient=3.30; 95% CI: 0.72, 5.89; p=0.019) (Table 4).

Sensitivity analysis

Since two studies were reported as having a high risk of bias and two other studies were considered as some concern in terms of risk of bias, these two groups of studies were combined in the sensitivity analysis. Consequently, the analyses revealed that the reported efficacy for MSC-CM in the studies having low risk of bias (SMD=2.1; 95% CI: 1.21, 3.20; p=0.001) did not significantly vary with that of studies having some concern and high risk of bias (SMD= 2.63; 95% CI: 0.42, 4.85; p=0.032). Moreover, the leave-one-out sensitivity analysis showed that eliminating none of

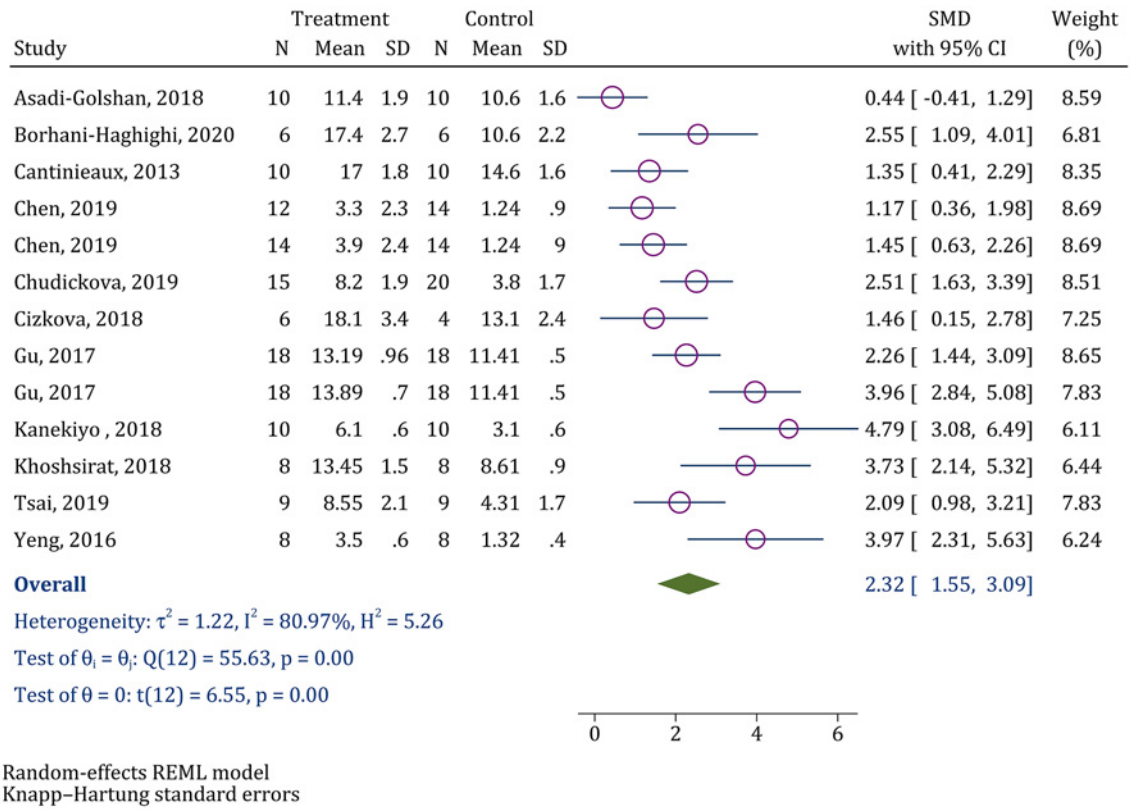

Figure 2. Forest plot for the effect of conditioned media on locomotor recovery after spinal cord injury.
SMD: Standardized mean difference; CI: Confidence interval

the articles significantly affected the pooled SMD (Fig. 3).

Publication bias

Egger's test revealed that there existed no publication bias regarding the efficacy of MSCs derived CM in motor recovery following SCI in animal models (p=0.780) (Fig. 4).

Discussion

Findings of the present meta-analysis revealed that MSC-CM administration promotes motor recovery in animal models following SCI. Since a 0.2 score for the effect size shows a weak efficacy, 0.5 score shows a moderate efficacy and a score of ≥0.8 shows a strong efficacy[28,29], our findings present a strong efficacy for MSC-CM treatment following SCI in animal models.

Several systematic reviews have been published on the efficacy of MSC-CM treatment on CNS repair, and the reported efficacy on motor function enhancement is attributed to the growth factors (such as Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), matrix metalloproteinase, Vascular endothelial growth factor (VEGF), and Hepatocyte growth factor (HGF)) secreted from these cells resulting in the repair and maintenance of the nervous tissue[30-33]. However, no systematic review has been published on the efficacy of MSC-CM on the brain or spinal cord tissue. The existing systematic review studies were found to be conducted on the administration efficacy of CM on bone regeneration (on animal and clinical studies)[34], pulmonary fibrosis[35], and lung disease[36]. All these studies have confirmed the efficacy of CM administration in tissue repair attributed this repair to the anti-inflammatory properties of the factors found in CM. The anti-inflammatory effects of CM prevent TNFa and IL-6 rises in the nervous tissue of the spinal cord, which may lead to tissue protection and promotes functional recovery following SCI[14].

Another nervous tissue protection, eventually causing functional recovery improvement, occurs as a result of angiogenesis. Angiogenesis provides oxygen and nutritional factors, preventing nervous tissue and regrowth of the axons[37,38]. Conversely, VEGF, as one of the key elements in CM, is essential in angiogenesis, besides its neuroprotective effects[39,40]. Osteopontin[41,42], fibroblast growth factor-binding protein[43,44], and matrix metalloproteinase-13[45], which are present in the CM, are also responsible for the initiation and progression of angiogenesis and consequently motor function recovery.

Besides the stimulants of angiogenesis, antiapoptotic factors are present in the CM, which prevent tissue destruction and protect the nervous tissue resulting in motor recovery following SCI. These factors include NGF (protecting sympathetic and sensory neurons)[46], TIMP-1 and CINC-3 (protecting motor neurons)[47] and BDNF (reducing astroglia scar formation)[48].

The findings of the present study demonstrated that MSC-CM administered volumes of more than 120 μL promote the

Table 3. Subgroup Analysis.

Subgroups	No. of experiments	SMD (95% CI)	P value	Heterogeneity (p value)
Severity of SCI				
Moderate	8	2.32 (1.36, 3.28)	0.001	76.98% (<0.0001)
Severe	5	2.40 (0.34, 4.45)	0.032	88.54% (<0.0001)
Cell source				
BMSCs	7	2.14 (0.92, 3.36)	0.005	79.64% (0.001)
Other	6	2.54 (1.16, 3.92)	0.005	83.50% (<0.0001)
Antibiotic				
Yes	9	2.57 (1.44, 3.71)	0.001	87.12% (<0.0001)
No	4	1.76 (0.92, 2.60)	0.007	0.00% (0.494)
Treatment protocol				
Single dose	11	2.17 (1.30, 3.04)	<0.0001	80.07% (<0.0001)
Multi dose	2	NA	NA	NA
Delivery type				
IT	6	1.68 (1.05, 2.31)	0.001	32.47% (0.205)
IP	3	3.22 (0.83, 5.61)	0.029	67.33% (0.035)
IV	2	NA	NA	NA
In situ	1	NA	NA	NA
Intraventricular	1	NA	NA	NA
Medium				
DMEM	11	2.41 (1.48, 3.34)	<0.0001	82.49% (<0.0001)
Other	2	NA	NA	NA
Type of graft				
Allogenic	7	2.71 (1.50, 3.92)	0.002	78.39% (<0.001)
Xenogeneic	6	1.88 (0.62, 3.14)	0.012	80.69% (0.001)
Delivery amount				
≤120 μL	7	1.49 (0.81, 2.18)	0.002	56.46% (0.032)
>120 μL	6	3.32 (2.20, 4.45)	0.001	56.67% (0.012)
Follow-up duration				
<8 weeks	8	2.57 (1.35, 3.79)	0.002	84.38% (<0.0001)
≥8 weeks	5	1.94 (0.74, 3.14)	0.011	67.15% (0.022)
Risk of bias				
Low risk	9	2.21 (1.21, 3.20)	0.001	84.48% (<0.001)
High risk and some concern	4	2.63 (0.42, 4.85)	0.032	72.72% (0.020)

BMSCs: Bone marrow mesenchymal stem cell; DMEM: Dulbecco's Modified Eagle's medium; IP: Intraperitoneal; IT: Intrathecal; IV: Intravenous; NA: Not applicable due to the limited number of included studies in the subgroup

Table 4. Multiple Meta-regression for Identification of the Source of Heterogeneity.

Variables	Coefficient	95% CI	P
Delivery amount			
≤120 μL	*Ref.*	*Ref.*	*Ref.*
>120 μL	3.30	0.72 to 5.89	0.019
Delivery type			
IT	*Ref.*	*Ref.*	*Ref.*
IP	−2.33	−5.93 to 1.28	0.171
Other	−1.89	−4.77 to 0.99	0.165
Antibiotic			
Yes	*Ref.*	*Ref.*	*Ref.*
No	−0.79	−2.56 to 0.99	0.329
Follow-up duration			
<8 weeks	*Ref.*	*Ref.*	*Ref.*
≥8 weeks	−0.38	−2.28 to 1.51	0.645

CI: Confidence interval; IP: Intraperitoneal; IT: Intrathecal; *Ref.*: Reference category

efficacy of the treatment in motor recovery following SCI. Nonetheless, although the studies administering higher doses of CM observed greater efficacies, their quality assessment revealed that all of the studies entered in the present meta-analysis lacked sample size calculations and optimal dose assessments. Thus, as a determinant in the process of translating the results into clinical applications[49], the mentioned quality shortcomings are considered to be among the limitations of the present study. Furthermore, as a disadvantage for the CM treatment in comparison with stem cell therapy, cytokines and growth factors present in the CM have shorter half-lives, resulting in multiple drug administrations when CM therapy is intended[50]. Thus, it is suggested that further animal studies be performed to determine the optimal administered dose.

The primary goal in animal studies is to evaluate the treatment efficacy, whereas in clinical studies, the first step to approve a treatment strategy is to determine the safety of the treatment. Therefore, as another limitation of the present

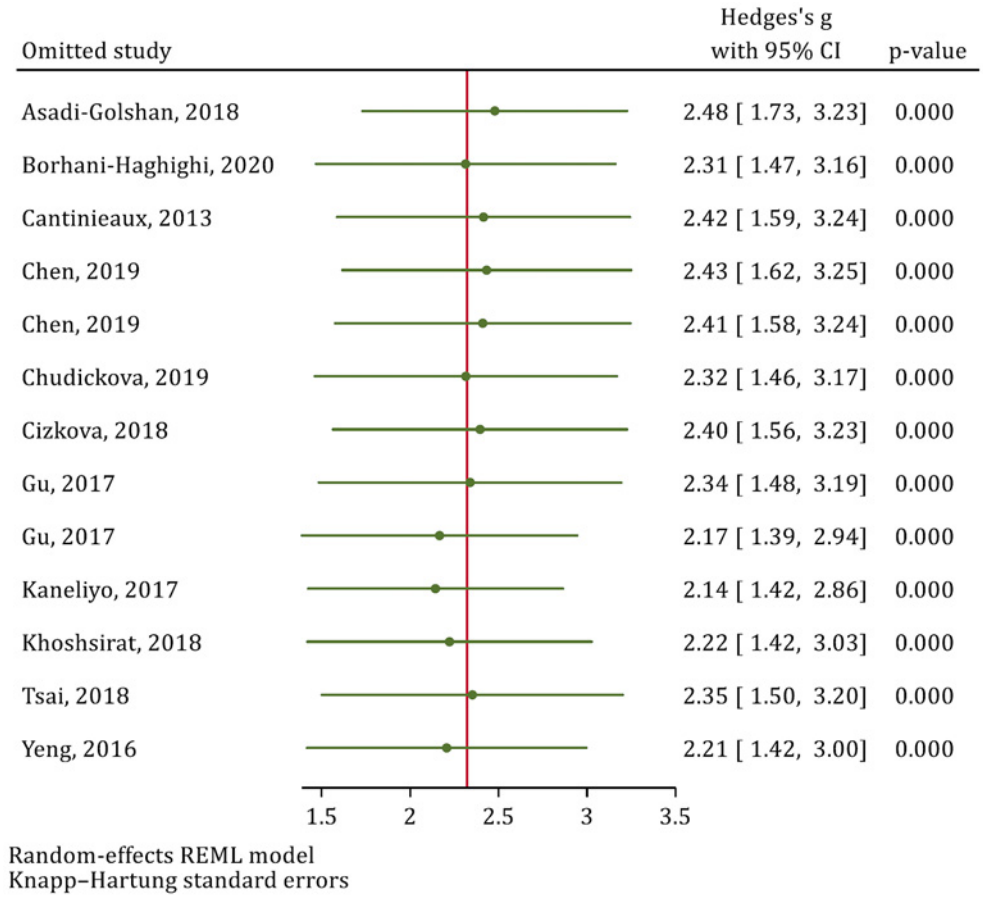

Figure 3. Leave-one-out sensitivity analysis to explore single study's effect on overall effect size.

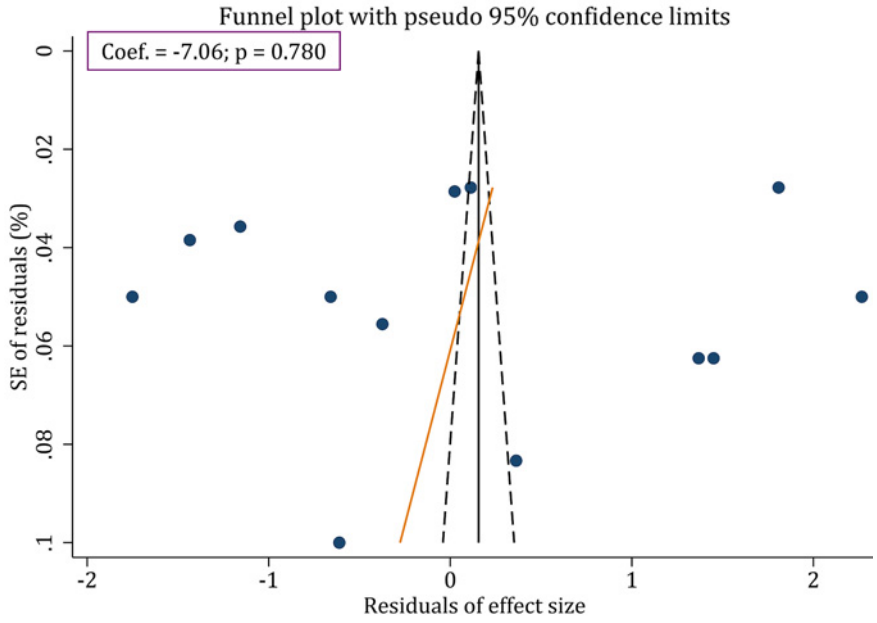

Figure 4. Funnel plot for assessment of publication bias in the efficacy of conditioned media on locomotor recovery after spinal cord injury.

study, the safety and adverse effects of the CM treatment were not evaluated in the animal studies included in our meta-analysis. Consequently, it is recommended that the safety of CM treatment be assessed in future studies. It is worth mentioning that no clinical trials exist regarding the application of MSC-CM in SCI, whereas clinical trials are on their way in the application of MSCs-CM in SCI, while clinical trials are on their way regarding the application of

CM in other conditions[51], as well as the application of MSCs in SCI[30,52]. This highlights the need for further animal studies and clinical trials for researchers to be able to investigate the clinical application and translation of this treatment strategy.

Conclusion

The findings of the present study revealed that MSC-CM administration promotes motor recovery in SCI animal models. The efficacy of this treatment strategy enhances in volumes higher than 120 μL. Since MSC-CM treatment has fewer limitations in comparison with MSC administration, the treatment can be considered as an alternative treatment approach in translational studies.

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