



Systematic Review

Mesenchymal Stromal Cells Derived Conditioned Medium in Pulmonary Fibrosis: A Systematic Review and Meta-analysis

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Abstract

Background: A definitive conclusion on the efficacy of mesenchymal stromal cells-derived conditioned medium (MSCs-CM) in pulmonary fibrosis has not yet been reached. Therefore, the present meta-analysis intends to investigate the efficacy of MSCs-CM administration on improvement of pulmonary fibrosis.

Methods: An extensive search was performed on the Medline, Embase, Scopus and Web of Science databases by the end of August 2019. Outcomes in the present study included pulmonary fibrosis score, lung collagen deposition, lung collagen expression, transforming growth factor β 1 (TGF- β 1) expression and interleukin-6 expression. Finally, the data were pooled and an overall standardized mean difference (SMD) with a 95% confidence interval (CI) was reported.

Results: Data from seven studies were included. Analyses showed that administration of MSCs-CM significantly improved pulmonary fibrosis (SMD = -2.36; 95% CI: -3.21, -1.51). MSCs-CM administration also attenuated lung collagen deposition (SMD = -1.70; 95% CI: -2.18, -1.23) and decreased expression of type I collagen (SMD = -6.27; 95% CI: -11.00, -1.55), type III collagen (SMD = -5.16; 95% CI: -9.86, -0.47), TGF- β 1 (SMD = -3.36; 95% CI: -5.62, -1.09) and interleukin-6 (SMD = -1.69; 95% CI: -3.14, -0.24).

Conclusion: The present meta-analysis showed that administration of MSCs-CM improves pulmonary fibrosis. It seems that the effect of MSCs-CM was mediated by reducing collagen deposition as well as inhibiting the production of inflammatory chemokines such as TGF- β 1 and interleukin 6 (IL-6). Since there is no evidence on the efficacy of MSCs-CM in large animals, further studies are needed to translate the finding to clinical studies.

Keywords: Conditioned medium, Lung fibrosis, Stem cells

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Introduction

Idiopathic pulmonary fibrosis is a chronic and progressive disease with an unknown etiology. The prevalence and incidence of idiopathic pulmonary fibrosis have been increasing in recent decades¹ and it accounts for about 20% of all interstitial lung diseases.² About 3 million people have been reported worldwide to have idiopathic pulmonary fibrosis, with its prevalence increasing with age.³

There is not a definitive treatment for pulmonary fibrosis. Initial studies have suggested the positive effects of corticosteroids and immunosuppressive agents in management of pulmonary fibrosis. However, systematic reviews have shown that there is insufficient evidence for the efficacy of these therapies.^{4,5} The current guidelines approve pirfenidone and nintedanib for treatment of

pulmonary fibrosis. However, the ideal therapeutic approach for management of pulmonary fibrosis is still under investigation.^{6,7}

Stem cells transplantation has been suggested as a therapeutic option for many conditions.⁸⁻¹⁰ A systematic review in 2015 showed that administration of mesenchymal stromal cells (MSCs) in animal models of pulmonary fibrosis improved fibrosis, decreased collagen deposition in lung, and reduced the expression of profibrotic chemokines.¹¹ Although preclinical studies have shown the benefit of MSCs transplantation, this treatment option has some limitations, including the possibility of tumorigenesis. In addition, circulating MSCs play a critical role in the pathogenesis of pulmonary fibrosis. For example, bone marrow-derived mesenchymal progenitor cells are able to differentiate into fibroblasts. This

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conversion to myofibroblasts is intensified in the presence of tumor growth factor β (TGF- β), which is elevated in pulmonary fibrosis.¹² On the other hand, lung resident MSCs secretome has anti-fibrotic effects.¹³ Therefore, caution should be exercised in the administration of MSCs to attenuate the progression of pulmonary fibrosis.

The use of conditioned medium as an alternative option to stem cell therapy has been suggested in some studies.^{14,15} Recent studies show that the paracrine effects of MSCs play a major role in treatment of acute and chronic conditions.¹⁶⁻¹⁸ Therefore, administration of MSCs-derived conditioned medium (MSCs-CM) may have all the effects of stem cell transplantation while omitting the side effects of stem cell administration. Preclinical studies have shown that administration of MSCs-CM could improve pulmonary fibrosis;¹⁹⁻²¹ however, no consensus has yet been made in this field. Therefore, the present systematic review and meta-analysis intends to investigate the efficacy of MSCs-CM administration in improving pulmonary fibrosis.

Materials and Methods

Study Design and Search Strategy

An extensive search of the Medline, Embase, Scopus and Web of Science databases was conducted by the end of August 2019. PICO in the present study was defined as pulmonary fibrosis (problem), administration of MSCs-CM (intervention), comparison with non-treated animals (comparison), and pulmonary fibrosis score and fibrotic and profibrotic markers (outcome), respectively.

Keywords related to “pulmonary fibrosis” in combination with words related to “mesenchymal stem cell derived conditioned medium” were used in the search databases. Systematic searches were performed for each database using proper Boolean operators and standard tags. The search query in Medline (via PubMed) is presented in Appendix 1. In addition, a manual search was conducted on Google, Google Scholar and bibliography of relevant articles to find additional studies.

Eligibility Criteria

We investigated all original studies published/accepted in a peer-reviewed journal on the efficacy of MSCs-CM administration in managing pulmonary fibrosis in animal models. There was no time or language limitation for the search. Exclusion criteria included lack of an untreated control group, conditioned medium derived from non-MSCs, MSCs-derived secretome, genetically engineered MSCs-CM, *in vitro* studies and review articles.

Data Gathering and Synthesis

Records obtained from the systematic search were screened by two independent reviewers. Potential relevant studies were then studied in full details and relevant articles were saved. Data were recorded by two researchers independently

in a pre-designed checklist. These data included first author's name, year of publication, number of animals, species, gender and age of animal, pulmonary fibrosis induction model, interval time between pulmonary fibrosis induction to treatment, source of MSCs, administration route, administered volume, type of graft (xenograft or allograft), follow-up duration and assessed outcome. Data were recorded as mean and standard deviation. Since in most animal studies, data are presented in graphs using plot digitizer software, mean and standard deviation were extracted. The use of this technique is a common method in animal meta-analysis studies.²²⁻²⁵

Outcome

First, we aimed to evaluate the effect of MSCs-CM on pulmonary fibrosis score, lung collagen deposition, collagen subtypes expression, TGF- β 1 expression and other profibrotic chemokines. Since meta-analysis should be performed when the outcome has been reported in at least two studies, we had to assess pulmonary fibrosis score, lung collagen deposition, type I collagen, type III collagen, TGF- β 1 expression and interleukin 6 (IL-6) expression.

Risk of Bias Assessment

Quality control of articles was performed based on SYRCLE's risk of bias tool – a 10-item risk assessment tool for pre-clinical studies.²⁶

Statistical Analysis

Data were analyzed using STATA 14.0 statistical software. Mean and standard deviation of the outcomes were recorded and a pooled effect size with a 95% confidence interval (CI) was reported for each study based on standardized mean difference (SMD) calculation. I^2 was used for assessment of heterogeneity and Egger's test for publication bias. Finally, subgroup analysis and meta-regression were performed to find the source of heterogeneity and to find the factors affecting the efficacy of MSCs-CM on the improvement of pulmonary fibrosis. $P < 0.05$ was considered as the level of significance in all analyses.

Results

Characteristics

Finally, 274 non-repetitive records were screened and data from 7 studies^{19,21,27-31} were included in the meta-analysis (Figure 1). Four studies were performed on rats while three were conducted on mice. Four studies used a bleomycin-induced pulmonary fibrosis model while three studies used other models such as silica-induced and radiation-induced pulmonary fibrosis. The time interval between pulmonary fibrosis induction to MSCs-CM administration ranged from 0 to 28 days. Four studies performed MSCs-CM transplantation within the first 24 hours after pulmonary fibrosis induction. The type of MSCs used was bone

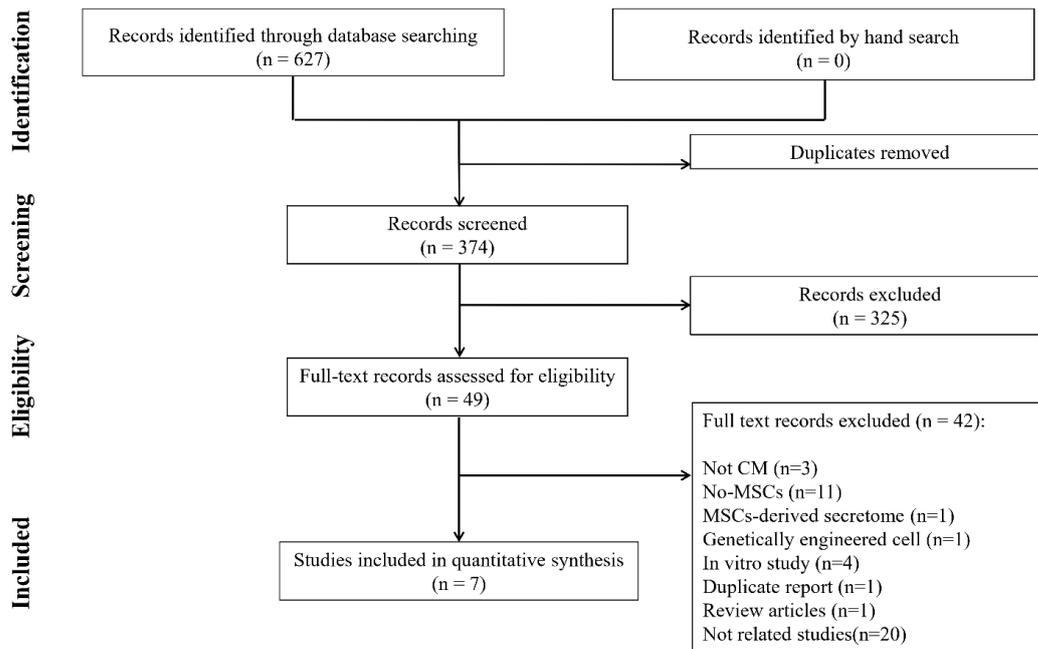


Figure 1. PRISMA Flow Diagram of Present Meta-analysis.

marrow-derived mesenchymal stromal cells (BMMSCs) in three studies, adipose tissue-derived mesenchymal stromal cells (ADSCs) in two studies, and amniotic-derived mesenchymal tissue cells (AMSCs) in two studies. MSCs-CM was injected intravenously in five studies and *in situ* in two studies. The transplanted volume of MSCs-CM ranged from 10 µL to 1000 µL. Follow-up duration varied between 11 and 28 days. Six studies examined fibrosis score, seven studies examined collagen deposition in lung tissue, four studies examined the expression of type I collagen, three studies type III collagen, three studies TGF-β1 expression and three studies IL-6 expression in lung tissue. Table 1 illustrates the characteristics of the included studies.

Risk of Bias Assessment

SYRCLE’s risk of bias tool showed random sequence generation, baseline characteristics of included animals, random housing, random outcome assessment, selective reporting, and other sources of bias in all eligible studies were of low risk. However, the risk of bias of allocation concealment and blinding of trial caregiver was unclear in six studies. Blinding of outcome assessment and incomplete outcome data in one study had high risk of bias (Table 2 and Figure 2A).

Publication Bias

The findings presented in Figures 2B and G show that according to Egger’s test, there was no publication bias in pulmonary fibrosis score (coefficient = 2.45; $P = 0.081$), collagen deposition (coefficient = 2.36; $P = 0.315$), expression of type I collagen (coefficient = 1.82;

$P = 0.495$), type III collagen (coefficient = 12.17; $P = 0.620$), TGF-β1 (coefficient = 5.31; $P = 0.198$) and IL-6 (coefficient = 0.61; $P = 0.919$).

Meta-analysis: Efficacy of MSCs-CM on Pulmonary Fibrosis Score

Five studies reported the efficacy of MSCs-CM on pulmonary fibrosis score. Meta-analysis of this section showed that administration of MSCs-CM significantly reduced the score of pulmonary fibrosis. In other words, administration of MSCs-CM improves pulmonary fibrosis (SMD = -2.36; 95% CI: -3.21 to -1.51; $P < 0.0001$) (Figure 3A). Subgroup analysis and meta-regression were performed despite mild heterogeneity between studies ($I^2 = 52.0%$; $P = 0.064$) (Table 3 and Figure 4).

Subgroup analysis indicated that the possible source of heterogeneity was animal species, type of MSCs and route of administration of MSCs-CM. It is worth noting that in all conditions, administration of MSCs-CM improved pulmonary fibrosis ($P < 0.001$) (Table 3).

Meta-regression also showed that none of the time interval between pulmonary fibrosis induction to MSCs-CM administration ($P = 0.722$), administered MSCs-CM volume ($P = 0.160$), number of MSCs for conditioned medium derivation ($P = 0.754$) and follow-up duration ($P = 0.920$) had any effect on the efficacy of MSCs-CM on pulmonary fibrosis score (Figure 4).

Meta-analysis: Efficacy of MSCs-CM on Lung Collagen Deposition

Six studies reported the efficacy of MSCs-CM on reduction of collagen deposition. Meta-analysis of this section

Table 1. Characteristics of Included Studies

Author; Year	Gender; Strain; Species; Age	Sample Size*	Method of LF Induction	Time Gap**	Type of MSCs	Cell Donor	Administration Route	No. of Cells	MSCs-CM Transplanted Volume (µL)	Type of Graft	FU (Day)	Outcome
Cargnoni; 2012	F; C57BL/6; Mice; 8-9 weeks	12 / 12	4 U/kg bleomycin; IT	0	AMSCs	Human	In situ	4 × 10 ⁵	10	Xenograft	14	Fibrosis score; Coll deposition
Cargnoni; 2014	F; C57BL/6; Mice; 8-9 weeks	9 / 35	4 U/kg bleomycin; IT	0	AMSCs	Human	In situ	1 × 10 ⁶	10	Xenograft	28	Fibrosis score; Coll deposition; TGF-β1; IL-6
Felix; 2019	M; Wistar; Rat; 8 weeks	10 / 10	1.5 U/kg bleomycin; IT	10	ADSCs	Equine	IV	1 × 10 ⁶	200	Xenograft	11	Fibrosis score; Coll-1; TGF-β1
Hansmann; 2012	NR; NR; Mice; Pups	7 / 7	Hyperoxia-induced	1	BMMSCs	Mice	IV	NR	50	Allograft	14	Coll deposition
Li; 2018	F; Wistar; Rat; Adult	8 / 8	50 mg/rat silica; IT	1	BMMSCs	Rat	IV	2 × 10 ⁶	1000	Allograft	30	Coll deposition; Coll-1; Coll-III; TGF-β1
Rathinasabapathy; 2016	M; SD; Rat; 8 weeks	10 / 10	2.5 U/rat bleomycin; IT	3; 7	ADSCs	Rat	IV	1 × 10 ⁶	100	Allograft	14	Fibrosis score; Coll deposition; Coll-1; Coll-III; IL-6
Zhang; 2018	M; SD; Rat; 6-8 weeks	6 / 6	50 mg/rat silica; IT	28	BMMSCs	Rat	IV	2 × 10 ⁶	100	Allograft	28	Fibrosis score; Coll deposition

ADSC, Adipose tissue derived mesenchymal stromal cell; AMSCs, Amniotic derived mesenchymal tissue cells; BMMSCs, Bone marrow derived mesenchymal stromal cell; CM, Conditioned medium; Coll, Collagen; F, Female; FU, Follow up duration; IL-6, Interleukin-6; IT, Intratracheally administration; IV, Intravenous; LF, Lung fibrosis; M, Male; MSCs, Mesenchymal stromal cells; MSCs-CM, Mesenchymal stromal cells derived conditioned medium; SD, Sprague Dawley; TGF-β1, Transforming growth factor beta 1.

*Number of untreated animal / Number of conditioned medium treated animals.

**Interval time between injury to treatment (day).

Table 2. Assessment of Risk of Bias in Included Studies

Criteria	Cargnoni; 2012	Cargnoni; 2014	Felix; 2019	Hansmann; 2012	Li; 2018	Rathinasabapathy; 2016	Zhang; 2018
Random sequence generation	+	+	+	+	+	+	+
Baseline characteristics	+	+	+	+	+	+	+
Allocation concealment	?	?	?	?	?	+	?
Random housing	+	+	+	+	+	+	+
Blinding of trial caregiver	?	?	?	?	?	+	?
Random outcome assessment	+	+	+	+	+	+	+
Blinding of outcome assessment	+	+	+	?	-	+	-
Incomplete outcome data	-	+	+	+	+	+	+
Selective reporting	+	+	+	+	+	+	+
Other source of bias	+	+	+	+	+	+	+

+, Low risk of bias; -, High risk of bias; ?, Unclear risk of bias.

showed that administration of MSCs-CM significantly decreased collagen deposition in animals with pulmonary fibrosis (SMD = -1.70; 95% CI: -2.18 to -1.23; $P < 0.0001$) (Figure 3B).

Subgroup analysis showed that differences in animal

species, pulmonary fibrosis induction model, type of MSCs, type of MSCs-CM administration, and type of graft (xenograft versus allograft) did not affect the efficacy of MSCs-CM in reducing collagen deposition in pulmonary fibrosis (Table 3).

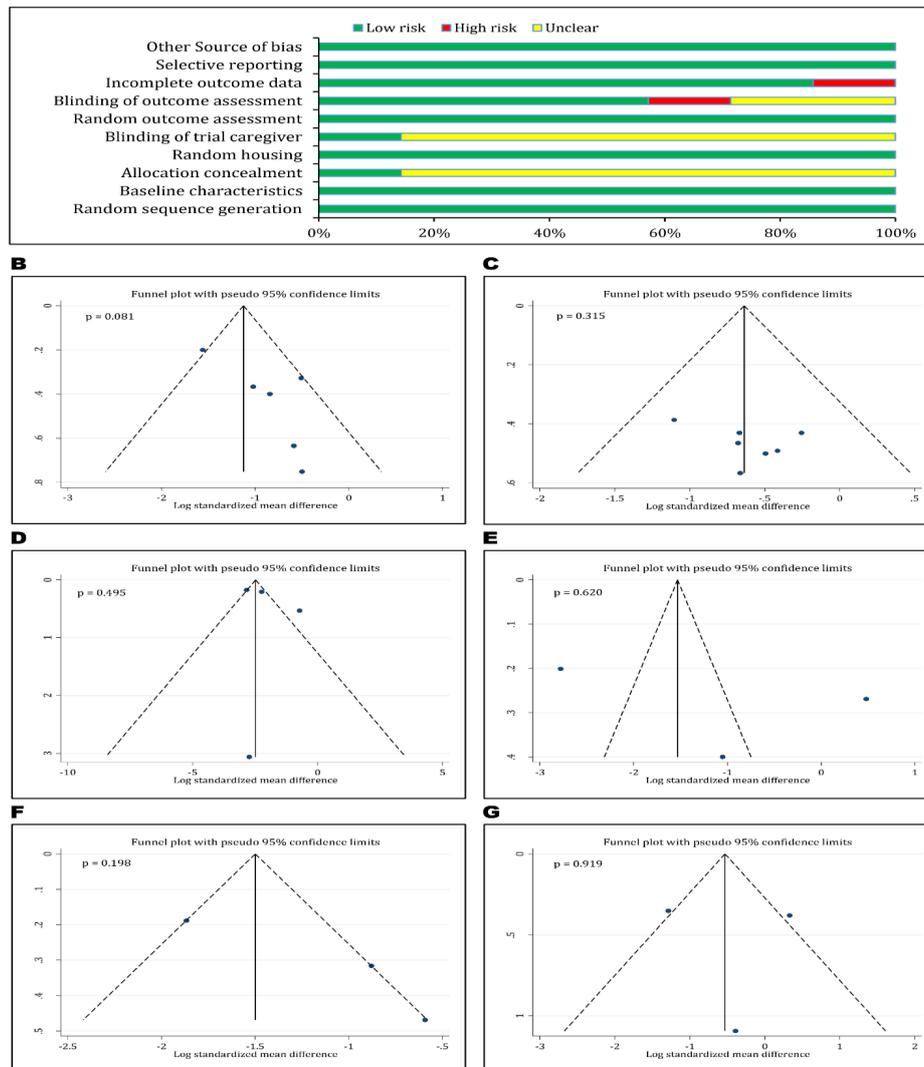


Figure 2. Assessment of Risk of Bias. A) publication bias of mesenchymal stromal cells derived conditioned medium on lung fibrosis score. B) lung tissue collagen deposition. C) lung tissue collagen type-I. D) and collagen type-III. E) expression, lung tissue transforming growth factor β 1 (TGF- β 1). F) and interleukin 6 expression. G) in lung fibrosis animal models.

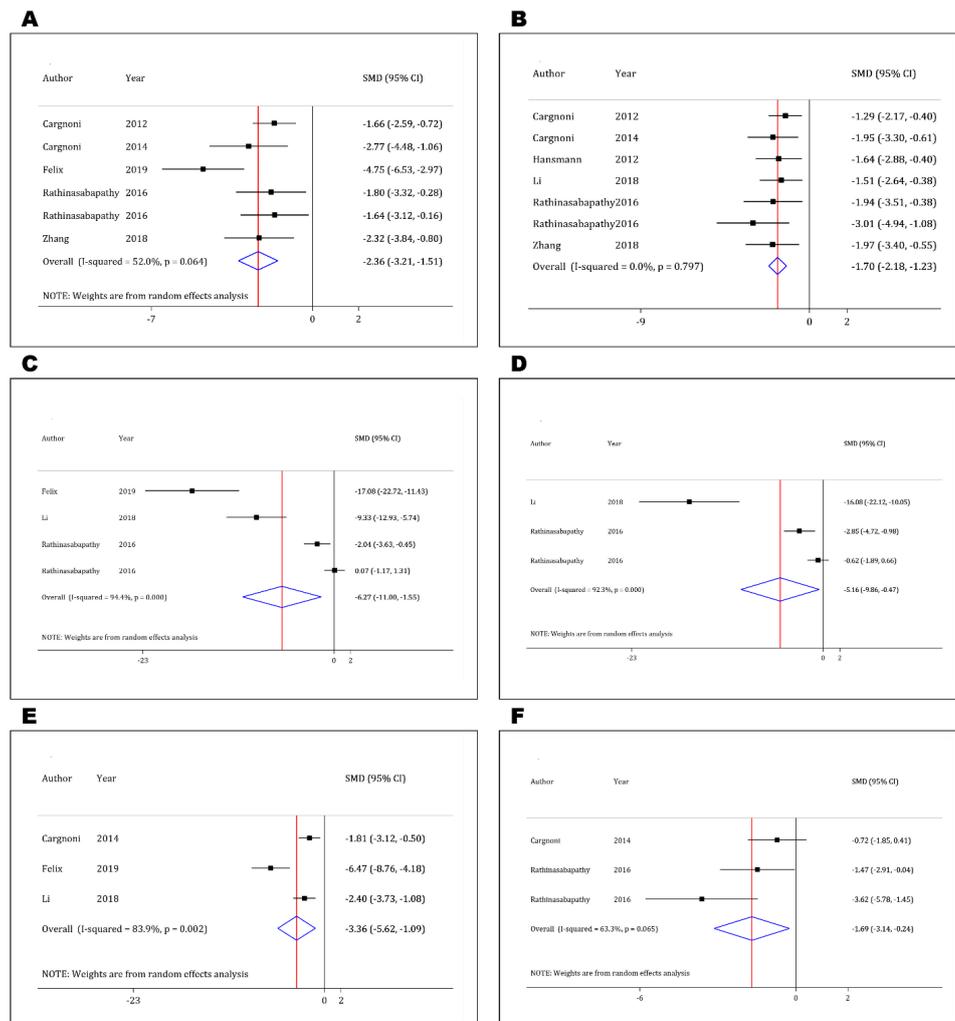


Figure 3. Forest Plots for the Effect of Mesenchymal Stromal Cells Derived Conditioned Medium on Lung Fibrosis Score. A) lung tissue collagen deposition. B) lung tissue collagen type-I. C) and collagen type-III. D) expression, lung tissue transforming growth factor $\beta 1$ (TGF- $\beta 1$). E) and interleukin 6 expression. F) in lung fibrosis animal models.

Meta-regression also showed that none of the time interval factors between pulmonary fibrosis induction to MSCs-CM administration ($P = 0.528$), administered MSCs-CM volume ($P = 0.814$), and number of MSCs for conditioned medium derivation ($P = 0.58$) as well as follow-up duration ($P = 0.867$) had any effect on the efficacy of MSCs-CM on reducing collagen deposition in the lung (Figure 5).

Meta-analysis: Efficacy of MSCs-CM on Type I Collagen and Type III Collagen Expression

Data from four and three experiments were entered to evaluate the efficacy of MSCs-CM on type I collagen and type III collagen expression, respectively. Analyses showed that administration of MSCs-CM decreased the expression of type I collagen (SMD = -6.27; 95% CI: -11.00 to -1.55; $P = 0.009$) and type III collagen (SMD = -5.16; 95% CI: -9.86 to -0.47; $P = 0.031$) after induction of pulmonary fibrosis (Figures 3C and D).

Meta-analysis: Efficacy of MSCs-CM on Lung TGF- $\beta 1$ and IL-6 Expression

Analyses on data from three studies showed that administration of MSCs-CM significantly reduced TGF- $\beta 1$ (SMD = -3.36; 95% CI: - 5.62 to -1.09; $P = 0.004$) and IL-6 (SMD = -1.69; 95% CI: - 3.14 to -0.24; $P = 0.022$) expression in lung tissue following pulmonary fibrosis (Figures 3E and F).

Discussion

The present meta-analysis for the first time collected and analyzed quantitative data on the effect of MSCs-CM on the pulmonary fibrosis in animal studies. Analyses showed that administration of MSCs-CM improved pulmonary fibrosis. The effect of MSCs-CM could be mediated by reducing collagen deposition and expression as well as inhibiting the production of inflammatory chemokines such as TGF- $\beta 1$ and IL-6.

Risk of bias was low in most studies, which was one of the strengths of the present meta-analysis. However, blinding

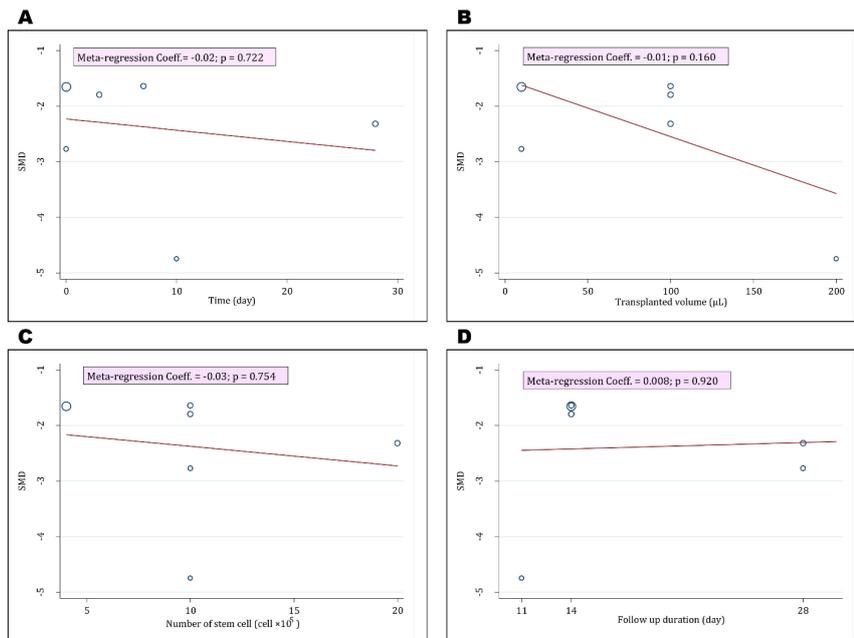


Figure 4. Meta-regression for the Effect of Time Interval between Lung Fibrosis Inductions to Mesenchymal Stromal Cells Derived Conditioned Medium (MSCs-CM) Administration. A) administrated MSCs-CM volume. B) number of MSCs for derivation of conditioned medium. C) and follow up duration. D) on the effect of MSCs-CM on lung fibrosis score.

of trial caregiver and allocation concealment details were not reported in six articles. Since the assessed outcome in the present study are histopathology assessment and protein expression assay, the blinding status seems to have little impact on the reported findings.

The reduction of fibrosis in the present study was evaluated by summarizing the data presented based on fibrosis score. This technique is a standard method for examining the rate of tissue fibrosis in the lung.^{32,33}

However, in interpreting the findings, the blinding status of outcome assessor is important. In the five studies included in this section, the risk of bias in the assessment of fibrosis score was low in four studies and unclear in one. Therefore, the findings are reliable.

Subgroup analysis showed that differences in the sources of MSCs did not affect the efficacy of MSCs-CM in improving fibrosis and collagen deposition. This finding is important because access to ADSCs cells is much easier

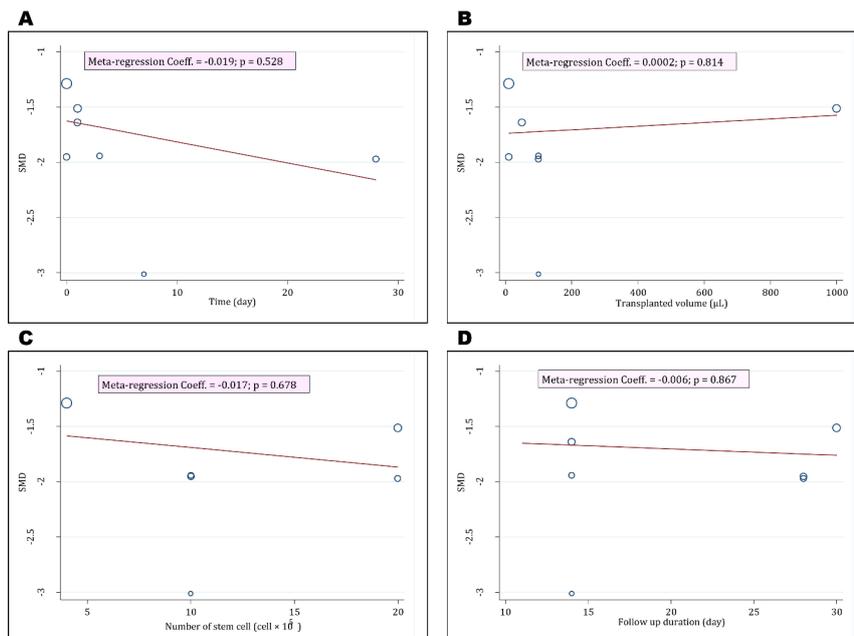


Figure 5. Meta-regression for the effect of Time Interval between Lung Fibrosis Inductions to Mesenchymal Stromal Cells Derived Conditioned Medium (MSCs-CM) Administration. A) administrated MSCs-CM volume. B) number of MSCs for derivation of conditioned medium. C) and follow up duration. D) on the effect of MSCs-CM on collagen deposition after lung fibrosis induction.

Table 3. Subgroup Analysis for Assessment of MSCs-CM Effect on Fibrosis Score and Collagen Deposition in Lung Fibrosis Animal Models

Subgroup	Number of Experiments	SMD (95% CI)	P Value	Heterogeneity	P Value
Fibrosis score					
Pooled analysis	6	-2.36 (-3.21 to -1.50)	<0.0001	52.0%	0.064
Animal species					
Mice	2	-1.98 (-2.96 to -0.99)	<0.0001	20.4%	0.262
Rat	4	-2.36 (-3.21 to -1.50)	<0.0001	63.8%	0.040
LF induction model					
Bleomycin	5	-2.39 (-3.44 to -1.36)	<0.0001	61.5%	0.034
Silica	NA	NA	NA	NA	NA
Type of MSCs					
AMSCs	2	-1.98 (-2.96 to -0.99)	<0.0001	20.4%	0.262
ADSCs	3	-2.67 (-4.53 to -0.81)	0.005	75.7%	0.016
BMMSCs	1	NA	NA	NA	NA
Route of administration					
<i>In situ</i>	2	-1.98 (-2.96 to -0.99)	<0.0001	20.4%	0.262
IV	4	-2.56 (-3.86 to -1.26)	<0.0001	63.8%	0.040
Type of graft					
Xenograft	3	-2.94 (-4.75 to -1.13)	0.001	78.4%	0.010
Allograft	3	-1.91 (-2.78 to -1.04)	<0.0001	0.0%	0.808
Collagen deposition					
Pooled analysis	7	-1.70 (-2.18 to -1.23)	<0.0001	0.0%	0.797
Animal species					
Mice	3	-1.53 (-2.16 to -0.90)	<0.0001	0.0%	0.705
Rat	4	-1.92 (-2.64 to -1.21)	<0.0001	0.0%	0.628
LF induction model					
Bleomycin	4	-1.73 (-2.36 to -1.09)	<0.0001	0.0%	0.418
Other	3	-1.67 (-2.39 to -0.95)	<0.0001	0.0%	0.883
Type of MSCs					
AMSCs	2	-1.49 (-2.23 to -0.75)	<0.0001	0.0%	0.418
ADSCs	2	-2.37 (-3.58 to -1.15)	<0.0001	0.0%	0.398
BMMSCs	3	-1.67 (-2.39 to -0.95)	<0.0001	0.0%	0.883
Route of administration					
<i>In situ</i>	2	-1.49 (-2.23 to -0.75)	<0.0001	0.0%	0.418
IV	5	-1.85 (-2.47 to -1.24)	<0.0001	0.0%	0.756
Type of graft					
Xenograft	2	-1.49 (-2.23 to -0.75)	<0.0001	0.0%	0.418
Allograft	5	-1.85 (-2.47 to -1.24)	<0.0001	0.0%	0.756

ADSCs, Adipose tissue derived mesenchymal stromal cell; AMSCs, Amniotic derived mesenchymal tissue cells; BMMSCs, Bone marrow derived mesenchymal stromal cell; CI, Confidence interval; CM, Conditioned medium; IV, Intravenous; LF, Lung fibrosis; MSCs, Mesenchymal stromal cells; MSCs-CM, Mesenchymal stromal cells derived conditioned medium; NA, Not applicable due to one study included in the subgroup; SMD, Standardized mean difference.

than other sources such as AMSCs and BMMSCs. In addition, their preparation and autograft transplantation are much more practical. Therefore, since the differences in cellular source have no effect on the conditioned medium's performance, it is recommended to use ADSCs derived conditioned medium in future studies.

In addition, a meta-regression analysis found that, unlike many chronic diseases, the interval between the occurrence of pulmonary fibrosis and the onset of treatment with MSCs-CM did not affect its efficacy. Therefore, it seems that even in the chronic phases of the disease, this treatment can be used to control the progression of pulmonary fibrosis. However, only one study identified a 4-week gap between the onset of pulmonary fibrosis and the onset of MSCs-CM administration. Therefore, the efficacy of MSCs-CM administration in the chronic phase of pulmonary fibrosis should be further investigated.

After MSCs-CM administration, type I and III collagen expression levels decreased significantly. Studies have shown that after pulmonary fibrosis, overexpression of type I and type III collagen is observed.^{34,35} These studies show that expression of type I collagen is also increased by TGF- β 1 expression. Therefore, administration of MSCs-CM seems to modulate collagen expression by reducing TGF- β 1 expression in lung tissue.

Most of the studies included in the present meta-analysis used the intravenous route to administer MSCs-CM. Subgroup analyses also showed that there was no difference between intravenous and *in situ* administration of MSCs-CM. This is an advantage for MSCs-CM because intravenous administration is one of the simplest routes of drug administration, whereas in many cases, intravenous administration of MSCs is associated with concern such as tumorigenesis.

In conclusion, the present meta-analysis showed that administration of MSCs-CM improved pulmonary fibrosis. Since intravenous administration of this therapeutic option has fewer risks than MSCs and its intravenous administration has a similar efficacy to *in situ* administration, the conditioned medium can be used in future studies as an alternative to MSCs transplantation. It seems that the effect of MSCs-CM was mediated by reducing collagen deposition as well as inhibiting the production of inflammatory chemokines such as TGF- β 1 and IL-6. In addition, it appears that ADSCs is the best source for preparation of conditioned medium.

Authors' Contribution

KMA, MH and MY: Study design. KMA, MY and FHRF: Data gathering. MH: Analysis. SHO and MIMG: Interpretation of the results. KMA and MY: Drafting of the paper. All authors: Revising the paper.

Conflict of Interest Disclosures

There is no conflict of interest.

Ethical Statement

Not applicable.

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