Iran J Public Health, Vol. 52, No.1, Jan 2023, pp.1-9

Review Article

Efficacy of Alveolar Type II Epithelial Cell Transplantation for Pulmonary Fibrosis: A Meta-Analysis

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(Received 21 Aug 2022; accepted 14 Oct 2022)

Abstract

Background: Cell transplantation is a promising therapeutic strategy for pulmonary fibrosis. In order to clarify the alveolar type II epithelial cell potential utility in the treatment of lung disease, we conducted a meta-analysis, to evaluate alveolar type II epithelial cells in animal models of lung injury and pulmonary fibrosis.

Methods: This review followed the recommendations from the PRISMA statements, Comprehensive retrieval method was used to search Embase, PubMed, Cochrane, Chinese Knowledge Infrastructure, VIP and Wanfang databases. A total of 7 studies and 286 model rats were included. Two researchers independently screened the identified studies, based on inclusion and exclusion criteria. All analyses were conducted using Review Manager V.5.3 software. The combined standard mean difference (SMD) and 95% confidence interval (CI) of data from the included studies were calculated using fixed or random-effects models.

Results: The analysis of three outcome indexes showed that the heterogeneity of the oxygen saturation group was high (I^2=85%), the lung weight group (I^2=64%) was close to moderate heterogeneity, and the lung hydroxyproline content group (I^2=0) was not heterogeneous.

Conclusion: Meta-analysis showed that transplantation of alveolar type II epithelial cells has beneficial effects, and no obvious adverse reactions. Alveolar type II epithelial cell transplantation can significantly reduce the intervention group and lung hydroxyproline content weight, improve the blood oxygen saturation, lung histopathology showed significant improvement in pulmonary fibrosis.

Keywords: Alveolar type II epithelial cells; Stem cells; Regenerative medicine; Treatment; Pulmonary fibrosis

Introduction

Pulmonary fibrosis (PF), especially idiopathic pulmonary fibrosis (IPF), is a chronic, life-threatening disease accompanied by shortness of breath and progressive deterioration of lung

function. Characterized by alveolar epithelial cell damage, remodeling of lung structure, abnormal accumulation of extracellular matrix, varying degrees of lung parenchyma inflammation and fibrosis, the median survival time of patients with IPF is estimated to be 2.5 to 3.5 years (1-4). IPF has a poor prognosis, with a mortality rate comparable to that of advanced tumors leading to respiratory failure and death (5, 6).

In recent years, pirfenidone, nintedanib and other drugs have improved the lung function of IPF patients, but neither of these methods has certain advantages in terms of mortality, and lung transplantation is often required (7-9). There is therefore an urgent need to find new and more effective treatments.

Regenerative medicine is an emerging and potential method of using cell therapy to treat target organ damage (10), In the search for cells that repair damaged lung tissue in IPF, alveolar type II epithelial cells (ATII) with stem cell properties have been evaluated in clinical trials (11).

ATII are endowed with important biosynthetic, secretory, metabolic, host defense, and repair or regeneration functions, which play a key role in maintaining alveolar homeostasis (12, 13). When alveolar type I epithelial cells (ATI) are injured, neighboring ATII are stimulated to proliferate and transdifferentiate into ATI (14, 15).

Several studies have shown that ATII have the ability to prolong the survival time of IPF models induced by bleomycin and silica (16-18). However, the dose, type, route, source and time interval of ATII in each study are different, and the final therapeutic effect is difficult to evaluate. Moreover, the optimal treatment of ATII is still unclear. Therefore, we collected data from all relevant studies and performed a meta-analysis to evaluate the efficacy of ATII therapy.

Methods

Search strategy

We followed the preferred reporting items for systematic evaluation and the Meta-analysis guidelines (4, 19). We searched the studies on the effect of ATII on pulmonary fibrosis animal model published in domestic and foreign biomedical journals from 2000 to 2021. All searches were conducted in 2021 and were in English and Chinese only. The search terms included "alveolar epithelial type II" and "pulmonary fibrosis." Comprehensive information was obtained by searching PubMed, MEDLINE, Cochrane, Chinese Knowledge Infrastructure, VIP and Wanfang database.

Inclusion criteria and study selection

Studies were first evaluated by reading titles and abstracts according to specified inclusion and exclusion criteria. The inclusion criteria were as follows: The study design focused on in vivo studies of intratracheal drug-induced pulmonary fibrosis in mice, interventions for endotracheal perfusion ATII, the study includes a sufficient number of samples (experimental group and control group), if the exposure is not on the lung injury induced by endotracheal drip or pulmonary fibrosis drug, if interventions including ATII, or if there is no assessment of hydroxyproline content in the lung, lung weight or blood oxygen saturation, then the study was excluded (20-22).

Data extraction and quality assessment

The data of the first author, year of publication, sample size of the experimental group and the control group, intervention content, intervention plan and outcome indicators were extracted and input by two researchers in an independent double-blind manner. According to the recommendations of evidence-based medicine research guidelines, the "risk of bias assessment" tool of Cochrane systematic review was adopted, the quality of the included studies was assessed on six criteria: randomization of the allocation method, hiding of the allocation scheme, blinding, integrity of the outcome data, selective reporting of the study results, and other sources of bias. In the statistical process, quality assessment is classified: 5 or more are low bias risk; 3 ~ 4 were moderate bias risk; Less than 3 items are considered high risk of bias (23).

Statistical analysis

The data was analyzed by Review Manager 5.3 software. Continuous data were collected, and mean difference (MD) or standardized mean difference (SMD) and 95% confidence intervals were used to assess treatment outcomes. Fixed effects model was used for data, and random effects model was used when heterogeneity was strong. I^2 was used to measure the heterogeneity of the included study, when $P>0.05$ or $I^2<50\%$, the data was considered as non-heterogeneity, and when $P<0.05$ or $I^2>50\%$, the data was considered as significant heterogeneity (24-26).

Results

Search results

After a comprehensive search, a total of 1671 studies were retrieved, among which 7 were finally included according to the previously described inclusion criteria (16,17,27-31). A schematic of the screening process and selection of studies for inclusion in the meta-analysis is provided in Fig. 1.

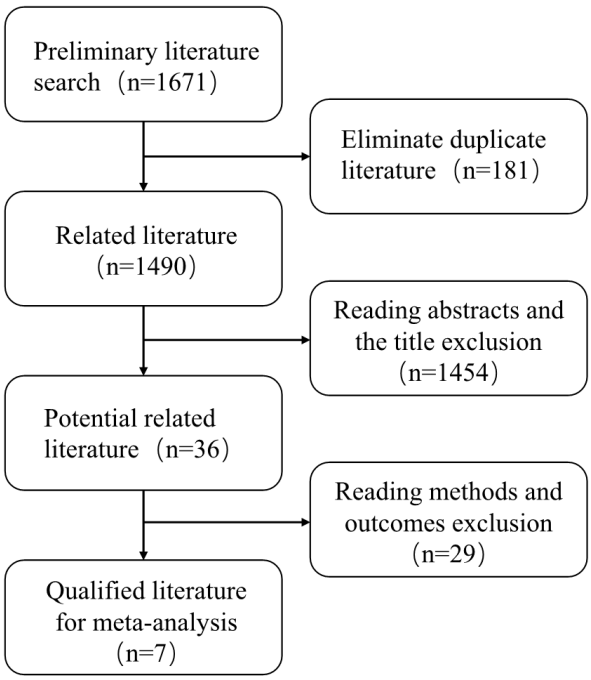

Fig. 1: Flow diagram of the selection of study for inclusion in the present Meta-analysis

Characteristics of included studies

A total of 286 model rats, including 7 experimental animal studies, were included in this analysis, of which 124 were treated and 162 were used as controls. All seven studies adopt endotracheal infusion drug induced pulmonary fibrosis, including a study using the acute lung injury induced by lipopolysaccharide model (30), a study using the model of silicon dioxide induced pulmonary fibrosis (17), the remaining 5 were used to research bleomycin was the induced fibrosis animal model. In three of the seven studies, interventions used human stem cell-induced alveolar type II cells (17, 29, 31), and in the remaining five studies all used animal alveolar type II cells (16, 27-30). Of the 7 studies, 4 reported lung hydroxyproline content (16, 27-29), 4 reported lung weight (16, 27, 28, 30), and 3 reported blood oxygen saturation (17, 29, 31) after alveolar type II transplantation, as shown in Table 1.

Table 1: Characteristics of the included studies

Reference	*Author*	*Year*	*Number of sample (T/C)*	*Intervention measures*		*Number of injected cells*	*Transplant time after modeling (days)*	*Outcome time (days)*	*Outcome indicator*
				T	C				
27	Raquel Guillamat-Prats	2014	8/8 rat	BLM+ ATII	BLM	$2.5x10^6$	14 days	21 days	b, c, d
28	Belen Alvarez-Palomo	2020	6/6 rat	BLM+ ATII	BLM	$3.0x10^6$	15 days	21 days	b, c, d
29	Dachun Wang	2010	6/22 mice	BLM+hES-ATII	BLM	$0.5x10^6$	1days, 2 days	10 days	a, b, c
30	Raquel Guillamat-Prats	2017	32/32 rat	(Hcl+lps)+ ATII	Hcl+lps	$2.5x10^6$	9 hours	72 hours	c, d
16	Anna Serrano-Mollar	2007	8/8 rat	BLM+ ATII	BLM	$2.5x10^6$	15 days	21 days	b, c, d
17	P. Spitalieri	2012	50/50 mice	SiO_2+hues-3-ATII	SiO_2	$2.5x10^6$	15 days	20 days	a, c
31	P. Spitalieri	2013	14/36 mice	BLM+hues-3- ATII	BLM	$2.5x10^6$	14 days	21 days	a, c

In all studies, ATII were administered via intratracheal instillation. T, treatment group; C, control group; ATII, Alveolar type II epithelial cells. A, Blood oxygen saturation ;b, hydroxyproline content; c, lung section electron microscope; d, lung weight

As for the quality methodological evaluation of the included literatures, none of the seven included literatures described the allocation method or blind method. There were 5 literatures with 3 scores, and the outcome indexes of the remaining 2 literatures were incomplete. In Fig. 2, the qualified literatures were "+", while the unqualified literatures were "-". Figure 3 is a statistical graph of the percentage of each item in the methodological evaluation.

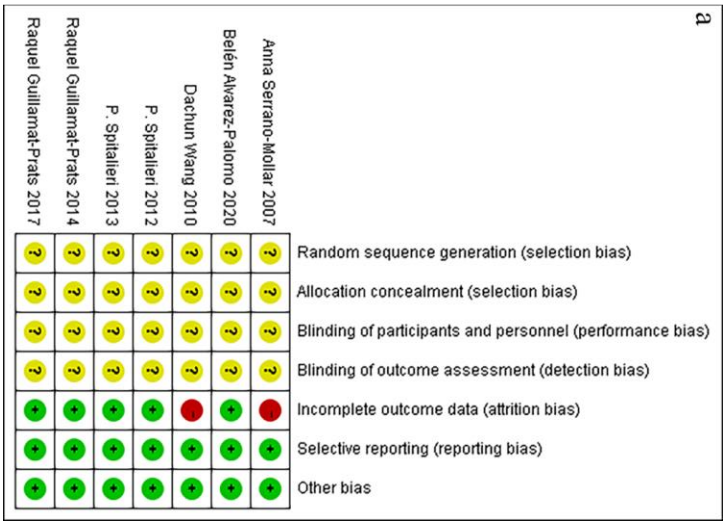

Fig. 2: Methodological Quality of Included Studies

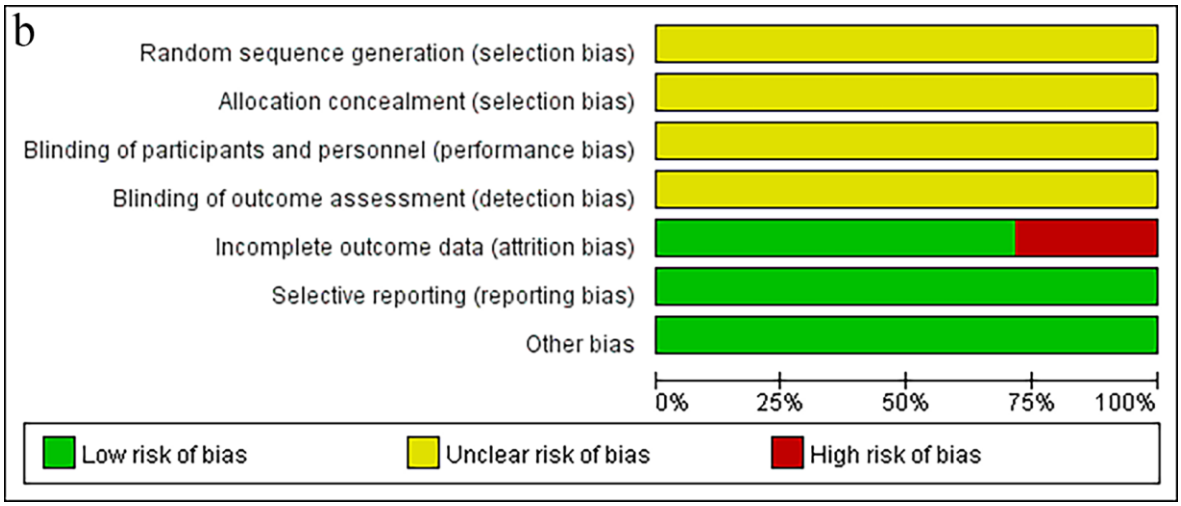

Fig. 3: The Distribution of the Methodological Quality of Included Studies

Improvement of oxygen saturation after ATII transplantation

The outcome index of 3 literatures all contained blood oxygen saturation (17, 29, 31), I^2=85%>50%, *P*=0.001<0.1, as shown in Fig.3. This analysis adopted the fixed effect model, with large heterogeneity, suggesting that the 3 literatures (17, 29, 31) selected in this study had large differences. As this analysis included 3 studies with a small sample size, no bias test was conducted. As can be seen from the meta-analysis forest map of blood oxygen saturation in Figure 4, the combined MD value of the three studies was 18.81, with a 95% confidence interval of 18.29 to -19.32, suggesting that in animals with pulmonary fibrosis, ATII transplantation could improve blood oxygen saturation in mice by 18.81%.

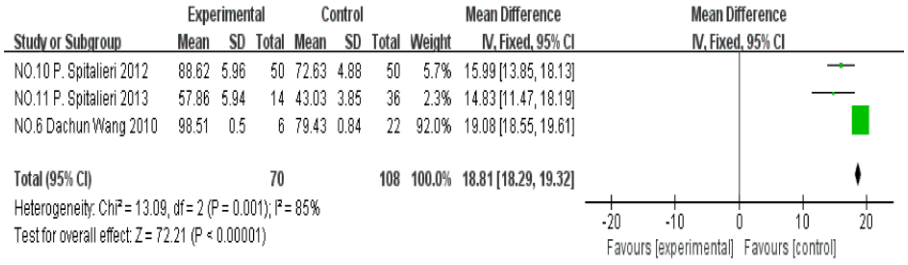

Fig. 4: Forest diagram compares ATII transplantation, and the result is blood oxygen saturation

Improvement in lung weight after ATII transplantation

In this analysis, lung weight was included in the outcome index of 4 literatures (16, 27, 28, 30). Among them, one study (30) established the model of lipopolysaccharide induced acute lung injury, and the intervention time was different from the other 3 studies, so the random effect model was used, as shown in Fig.5a, I^2=64%>50%, *P*=0.04<0.1. This suggests that there is heterogeneity among the four literatures (16, 27, 28, 30) selected for this study. The combined SMD value of four studies was -4.37 with a 95% confidence interval of -5.12 — -3.62, suggesting that ATII transplantation therapy can reduce lung weight in mice with pulmonary fibrosis.

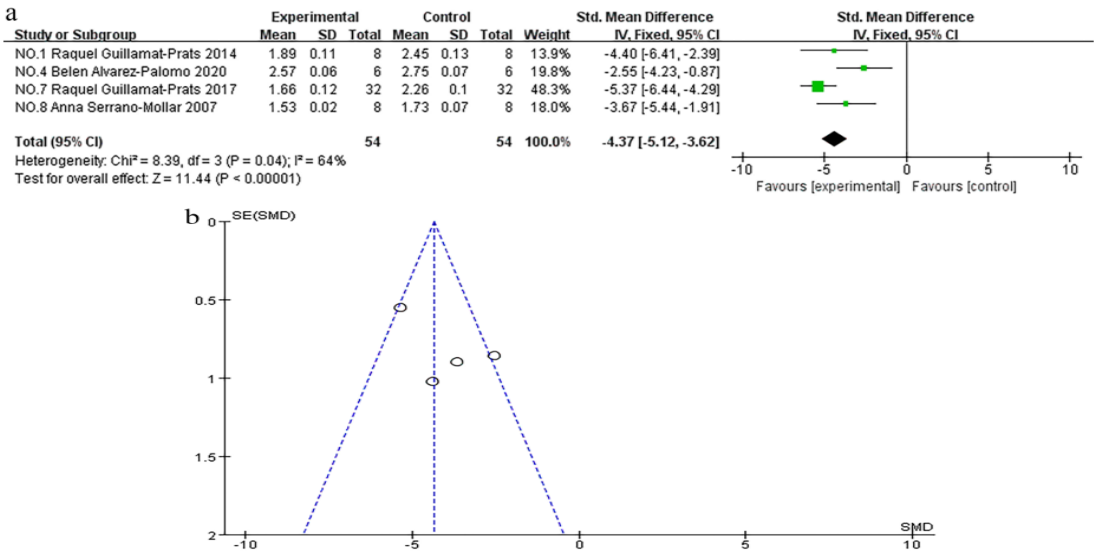

Fig. 5: **a:** Forest map compared ATII transplantation, and the result was lung weight. **B:** Funnel diagram of ATII therapy with lung weight as the outcome

Improvement of hydroxyproline content in ATII after transplantation

In this study, the outcome index of 3 literatures contained hydroxyproline content (16, 27, 28), and fixed effect model was adopted. As shown in Fig. 6. 6, I^2=0>50%, *P*=0.83>0.1, indicating that there was no heterogeneity among the 3 literatures (16, 27, 28) selected in this study. Funnel plot analysis showed a small difference between the groups, and the combined MD value of the three studies was -2.30, with a 95% confidence interval of -2.57 — -2.04, suggesting that ATII therapy can reduce the amount of hydroxyproline in the lungs by 2.3mg in animals with pulmonary fibrosis.

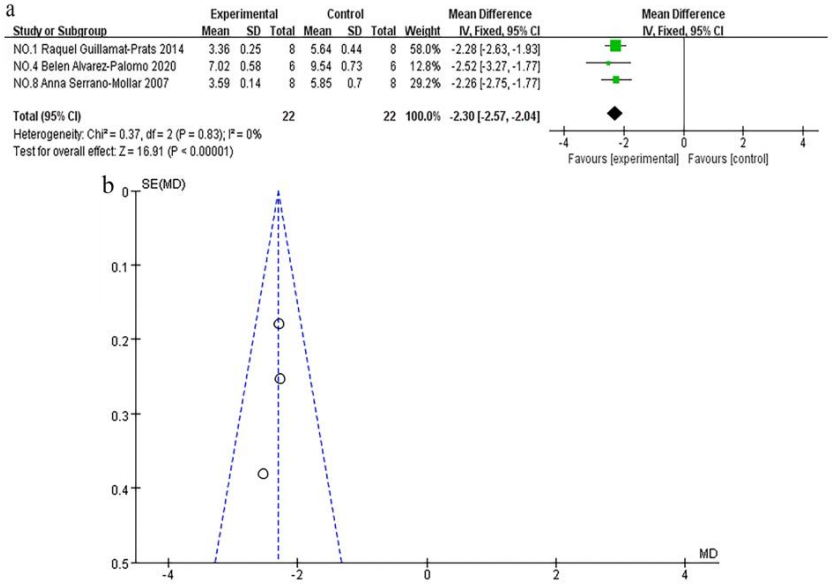

Fig. 6: **a:** Forest map compared ATII transplantation, and the result was hydroxyproline content. **b:** Funnel diagram of treatment of ATII with hydroxyproline content as the outcome

Discussion

This meta-analysis evaluated the efficacy of ATII therapy in a preclinical model of pulmonary fibrosis. Overall, the results of our meta-analysis suggest that ATII treatment can improve pulmonary fibrosis. In the seven studies. The main indicators of pulmonary fibrosis, such as hydroxyproline content and blood oxygen saturation, were quantitatively analyzed. ATII treatment significantly increased blood oxygen content and significantly decreased hydroxyproline content in PF animal models, indicating the potential of ATII transplantation in PF preclinical studies. This meta-analysis included animal models of PF and lung injury induced by bleomycin, silica, and lipopolysaccharide, providing more possibilities for ATII therapy in preclinical studies of PF.

In the field of regenerative medicine, animal studies are relevant for clinical use to evaluate the safety and efficacy of ATII therapy (32). Only one clinical trial has investigated the potential benefits of ATII transplantation in patients with IPF, and the results of existing clinical studies show that ATII endotracheal transplantation is safe, well tolerated, and has no significant adverse effects (11). Although ATII treatment is one of the cells that is generally safe and promising to slow disease progression in these preclinical trials, the optimal modality of ATII treatment remains unclear. We investigated the efficacy of different cells and found that the current more common use is bone marrow mesenchymal stem cells for pulmonary fibrosis (33). However, ATII is more readily available in a larger cell population than bone marrow mesenchymal stem cells. Our study reports that ATII also performs well in the treatment of pulmonary fibrosis. But due to the limited research available, more rigorous ATII treatment studies are needed to validate these findings.

We sought to explore heterogeneity across different design projects, including different cell types, injection doses, time intervals, and fibrosis types, we found that ATII dose may contribute to heterogeneity. The differences in pathological characteristics of PF models in many aspects may be responsible for the different therapeutic effects. Migrating ATII plays an important role in injury repair through differentiation in immunomodulatory and anti-inflammatory roles.

The SYRCLE Risk of Bias tool was used to assess the translational potentials of ATII therapy. None of the seven studies included in this analysis were assessed as having a low risk of bias. Although most studies attempt to avoid various biases, few researchers attempt to present their protocols. Therefore, we were unable to estimate effect sizes in subsequent studies based on this tool. The low quality of the methods is mainly due to the lack of double blindness, selective reporting, and incomplete outcome data. This meta-analysis highlights common problems and suggests an urgent need for higher methodological quality at the time of publication. We recommend that future studies related to ATII-based PF therapy should follow the SYRCLE risk of Bias tool to design future preclinical studies and reduce internal bias.

First, this meta-analysis includes different PF models and recently published high-quality studies. Secondly, we conducted a systematic literature search and comprehensive data collection, which can improve the accuracy of our research results. Third, the main results regarding hydroxyproline content and blood oxygen saturation analysis can provide important insights for future studies.

However, our study has some limitations. First of all, funnel plot test found that this meta-analysis was biased in the analysis of blood oxygen saturation. Second, current studies mostly focus on the effect of ATII after injury. Whether ATII treatment can produce long-term therapeutic effect on the remission of pulmonary fibrosis is worthy of further exploration. The success of ATII therapy depends in part on sufficient numbers of cells reaching the target organ and appropriate timing of injection. In studies, the effective dose of ATII treatment was usually 2.5×10^6 per 200 g of rats. Transplantation of ATII is more effective when fibrosis is established than in the early stag-

es of fibrosis, and future experiments are needed to further verify the antifibrotic effects of ATII.

Conclusion

This study is the first meta-analysis to evaluate the efficacy of ATII in the treatment of pulmonary fibrosis. ATII is effective in the treatment of pulmonary fibrosis in animal models, which provides an important basis for future translational clinical research. In the future, large-sample, prospective, double-blind, randomized controlled trials are needed to demonstrate the safety and efficacy of ATII in the treatment of IPF.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NSFC) [grant numbers 81602893, 81872575], Natural Science Foundation of Shandong Province [grant numbers ZR2021MH218], Innovation Project of Shandong Academy of Medical Science and Academic promotion programme of Shandong First Medical University [grant number 2019QL001].

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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