

Mesenchymal Stem Cells-Based Clinical Trials in ARDS

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ABSTRACT

Therapeutic interventions in acute respiratory distress syndrome (ARDS) primarily depend on lung-protective strategies, as no disease-modifying treatment has become available. In recent years, mesenchymal stem cells (MSCs) have been emerged as a new therapeutic option for ARDS, while among the early phase clinical trials, several studies showed tolerability and safety rather than efficacy of the MSCs. Here we summarized the results of published clinical studies on MSCs treatment in ARDS and discussed some approaches in improving the clinical trial design, aiming to help to enhance the implementation of cell-based therapy on ARDS.

Keywords: Stem cells, Acute lung injury, Therapy, Clinical research, Review

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is a severe clinical condition which may be triggered by various pathologies such as trauma, pneumonia and sepsis, and characterized by excessive inflammation in the lungs [1]. Despite fifty years of research, therapeutic interventions in ARDS remain primarily limited to supportive strategies, as no disease-modifying treatment has yet become available [2].

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into a variety of cell types [3]. Based on favorable results in preclinical models, MSCs can modulate the immune response and enhance recovery from lung injury, thus MSCs have been emerged as a new therapeutic option for ARDS [4]. Encouragingly, case reports suggested promising therapeutic potential [5,6]. While among the early phase clinical trials, several studies showed high tolerability than efficacy of the cells. Here we reviewed the results and designs of published clinical trials of MSCs in ARDS, trying to explore optimal clinical trial designs, aiming to help to collect more compelling evidence in new interventions.

RESULTS OF THE PUBLISHED CLINICAL TRIALS

Safety

Since the first clinical application of MSCs in 1995, MSCs have appeared to be well-tolerated with no cell infusion-related evidence of severe adverse effects (SAEs) in trials of various conditions [7,8]. Similar results on safety were seen in ARDS clinical trials.

The first clinical case of human umbilical cord MSCs (HUC-MSCs) therapy in ARDS was documented in 2012 [9]. Five patients with ARDS caused by acute paraquat poisoning received conventional treatment plus HUC-MSCs at a dose of 1×10^6 cells/kg body weight (BW) by I.V. once a day for five consecutive days. No adverse reactions were presented in the HUC-MSC group, and almost all the major organs function showed normal in re-examination, except for one case of incompletely absorbed shadow in the lung from CT scan.

In the first completed clinical trial published in 2014 using adipose tissue-derived MSCs (AT-MSCs) to treat ARDS [10], patients with ARDS randomly received one intravenous dose of 1×10^6 allogeneic AT-MSCs/kg BW in 100ml normal saline or 100ml normal saline as control. One patient from each group developed diarrhea and resolved within 48 hours. One patient in MSCs group presented with rash and resolved spontaneously. One patient in MSCs group died of multiple organ failures while two patients in placebo group respectively died of multiple organ failure or sepsis. The deaths were considered to be related to the preexisting

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disease processes but not the MSCs used in the study.

Bone marrow-derived MSCs (BM-MSCs) are the most commonly used stem cell types. In a dose-escalation clinical trial published in 2015 [11], JG Wilson et al. demonstrated that a single dose of allogeneic BM-MSCs by I.V. infusion was well tolerated in patients with moderate to severe ARDS. There was no pre-specified infusion associated events. SAEs were observed in three patients: two patients expired more than seven days after the MSC infusion, and one patient got multiple embolic infarcts that thought to have occurred prior the MSC infusion based on MRI results. None of these SAEs were thought to be related to MSC infusion.

Efficacy

From the studies above, the treatment of ARDS by MSCs showed good safety suggesting the possibility of MSCs clinical application, but the effectiveness evaluation results were divergent.

During the HUC-MSCs therapy study in ARDS caused by acute paraquat poisoning, the HUC-MSCs group showed significantly lower maximum SOFA scores and lung injury scores (LISs) than the control group after treatment [9]. More encouragingly, all the five patients in the HUC-MSCs group survived, while in the control group, there was only one patient out of eight survived.

In Zheng G, et al. study, AT-MSCs treatment significantly improved the patients' PaO₂/FiO₂ ratio from baseline and decreased serum SP-D levels at day 5 than those at day 0, suggesting that the MSCs may be effective in reducing epithelial cell injury, while the PaO₂/FiO₂, the assessment of hospital indices and other serum biomarkers did not reveal significant differences between MSCs and placebo groups [10].

JG Wilson et al. demonstrated changes in LIS and SOFA score with the high dose of BM-MSCs (10 million cells/kg BW) compared to lower doses [11]. However, this difference was not statistically significant between groups. Median levels of IL-6, RAGE, and Ang-2 levels all decreased between baseline and day three, while these markers are known to decline over time in patients with ARDS treated with low tidal volumes. Thus, without a matched control group, we cannot conclude that the observed biomarker changes were related to MSC therapy.

ISSUES ON THE CLINICAL TRIAL DESIGN

It is critically necessary to observe clear and significant clinical benefit in early clinical trials since the increased heterogeneity of populations and diseases in phase III-IV trials will often weaken the significance of minor benefits in early trials [12]. But the complex biological activities of stem cells and various mechanisms of ARDS pathogenesis have brought difficulties in clinical trials. Various biases

caused by inappropriate design, over-widened or unduly narrowed inclusion/exclusion criteria, improper interventions, as well as inadequate observation endpoints pre supposed or results interpretation; each will further impede our seeking for the scientific truth. Here we listed a selection of problems and challenges in clinical trial designs on MSCs therapy in ARDS, hoping to contribute to optimized research projects.

Clinical Protocols

Randomized controlled trials (RCTs) are recognized to provide more explicit proof of impact since RCTs are possible to avoid all kinds of bias and balance confounding factors, in which randomization, control, and blinding are considered as the essential principles [13].

Randomization, a core principle in the RCT, can reduce confounding by equalizing independent variables that have not been accounted for in the experimental design [14]. In the published clinical trials above, two of them didn't use random allocation procedure [9,11], and the only experiment using randomization didn't describe the exact randomly allocating method, so that the statistical power would be limited and the judgment of the results may be affected.

Control can determine how much benefit of the subjects regarding safety and efficacy come from experimental agents. In the dose-escalation clinical trial, the mortality rate was compared with the published general mortality rate, and the clinical outcomes and plasma biomarker levels were compared between different dose groups or using self-control [11]. Because of the variation in mortality rates and therapeutic efficiency among hospitals at different times, a placebo control or standard therapy control is highly recommended.

Blinding can effectively avoid the biases caused by subjective factors in evaluations. In Liu, W.W.'s study with all the five patients surviving in the HUC-MSCs and only one out of eight patients surviving in the control group, either of the researchers or patients were blinded [9]. Therefore, the reliability of the excellent curative effect might be reduced to a certain degree.

Enrollment criteria

Efficacy trials with well-defined and homogeneous populations are more probable to produce a clinically meaningful and statistically significant effect [15]. Among the registered nine clinical trials on stem cells in ARDS, seven studies enrolled all-cause ARDS patients (**Table 1**). Since ARDS is a heterogeneous clinical syndrome which can result from multiple conditions, it is likely that the underlying pathophysiology may be entirely different [16]. This may be one of the reasons why some clinical trials failed to show improvement in overall survival or various physiological parameters. Besides, Calfee et al. have identified a hyper-inflammatory phenotype of ARDS with

higher mortality using clinical and biologic data [17]. When applied to patients in earlier trials, these patients might be more likely to benefit from MSCs therapy.

Table 1: Registered clinical trials of stem cells in ARDS

NCT number	Study Design	Interventions	Disease Conditions	Status / Estimated Completion Date
NCT01775774 [11]	Multi-center, open-label, dose-escalation phase I clinical trial	Allogeneic BM-MSCs in 100ml by I.V. . 3 patients received 1million cells/kg BW; 3 patients received 5 million cells/kg BW; 3 patients received 10 million cells/kg BW.	Moderate to severe ARDS	Completed
NCT01902082 [10]	Single-center, randomized, double-blind, placebo-controlled phase I clinical trial	6 patients received one dose of 1 million allogeneic AT-MSCs/kg BW in 100ml normal saline and 6 patients received 100ml normal saline; all by I.V.	Moderate to severe ARDS	Completed
NCT02095444	Single center, open label phase I-II clinical trial	Menstrual blood stem cells 10 million cells/kg BW by I.V. for 4 times during two weeks	H7N9 virus infection caused ARDS	Unknown / December 2016
NCT02097641	Randomized, double-blind placebo-controlled phase II clinical trial	10 million BM-MSCs/kg BW or Plasmalyte A placebo in a 2:1 randomization scheme	Moderate to severe ARDS	Recruiting / February 2018.
NCT02112500	Open label phase II clinical trial	Autologous BM-MSCs, by I.V.	ARDS	Recruiting / December 2016
NCT02444455	Open label, controlled prospective phase I-II clinical trial	0.5 million HUC-MSCs/kg BW by I.V. once a day,a total of three times.	ARDS	Unknown / December 2017
NCT02611609	Randomized, quadruple blind, phase I/II Study	MultiStem or placebo	Moderate to severe ARDS	Recruiting / November 2018
NCT02804945	Randomized, double-blind phase II clinical trial	One dose of 3 million cell/kg by I.V.	Moderate ARDS includes patients with malignancies	Recruiting / February 2019
NCT03042143	Open label, dose escalation, phase I trial followed by a randomized, double-blind, placebo-controlled phase II Trial	HUC- CD362 +ve MSCs, Placebo Comparator: Plasmalyte.	Moderate to severe ARDS	Not yet recruiting / January 2022

Interventions

Cell origin, dosage, delivery route, and cell quality control, are all critical to the therapeutic effect.

Stem cells from different sources have various characteristics. AT-MSCs showed more potent immunomodulatory effects and greater proliferation capacity over than BM-MSCs [18], while umbilical cord blood-derived MSCs showed higher proliferation capacity than BM-MSCs and AT-MSCs [19]. And there are other optional stem cells --the endogenous lung stem cells seem to can integrate better into injured lung tissue [20], and endothelial progenitor cells appear to have therapeutic effects via differentiation into the endothelium of the damaged vascular site [21]. Which cell type or mixed multi-stem cells are more suitable for ARDS treatment remain to be determined by evidence from more preclinical and clinical research.

In the use of MSCs for ARDS treatment, whether there is a dose effect or a therapeutic ceiling by the safety concern remain unclear. In ARDS models of rodents, the effective administration dose is about $20\sim 30 \times 10^6$ cells/kg [22]. And in clinical applications above, the dose of MSCs ranged from 1 to 10×10^6 cells/kg. As many believe that higher doses will give enhanced or prolonged response, besides higher dose in the phase 1 trial by JG Wilson et al. was well tolerated and seemed to have more efficiency [11], the phase 2 study will use the high dose of 10×10^6 cell/kg [23], which results will be promising. And perhaps equally importantly, since the kinetics of an MSC graft is transient with a half-life

of approximately 24 h [24], it is unclear whether a second infusion of MSCs is needed.

The pathologic hallmark of ARDS is diffuse alveolar damage [1], but it is difficult to distribute the cells uniformly in lung tissue by intratracheal injection, and the intravenous route of a large amount of cell suspension may be more practical for clinical application in hypoxemic ARDS patients. However, for patients with bacterial infection, the intrapulmonary delivery of MSCs may be more efficient to enhance their antimicrobial activity through the secretion of antimicrobial peptides. Besides, Qin et al. found that MSCs delivered by intra-pleural delivery can prolong MSCs survival to at least one month [25]. Although promising, the optimal delivery route needs further investigation [22].

Besides, there are challenges in cell preparation including production and cryopreservation methods which may induce changes in cells function. In one study the viabilities of the given cells were only about 56% [11]. Furthermore, cell viability assays couldn't provide data regarding the potency of the cells. It was recommended that the cell lysates of MSC should be tested for potency by measuring some paracrine factors [2]. And it is necessary to establish a quantitative criterion for quality control of stem cells therapy, in cell characterization, cell viability and potency, bacterial and viral detection, etc.

Study Outcomes

Along with the complicated mechanisms of stem cell therapy, we need more comprehensive evaluations on the impact of MSCs treatment rather than mortality only.

Efficacy endpoints proposed in the ongoing phase II clinical trial exhibited an excellent example [23], in which respiratory efficacy endpoints (LIS, the PaO₂/ FiO₂ ratio, arterial blood gas measurement and chest radiograph), systemic efficacy endpoints (SOFA score, ventilator-free, ICU-free, vasopressor-free, organ failure free days and 60-day mortality), as well as all aspects of biologic measurements (inflammation indices, indexes of epithelial/ endothelial injury, analysis on MSCs paracrine activity, and other main organs injury). For MSCs paracrine activity measurements, in addition to angiopoietin-1 and keratinocyte growth factor mention in this study, we suggest that it is better to detect the quantity and function of the extracellular vesicles produced by stem cells in BAL and serum as well [26,27]. And the follow-up period of this study was about to be extended to 12 months, which will provide a longer-term effect of MSCs therapy.

CONCLUSION

Though questions and concerns remain, stem cell-based therapies are undergoing rapid development and offer promise for the treatment of ARDS. We expect more clinical trials with elaborate experimental design based on support from basic research, hoping that feasible and effective MSCs therapy can eventually change the treatment diagram of ARDS.

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